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89. SEASONAL VARIATION OF THE PERCENTAGE BUTTERFAT CONTENT OF MILK

AN EXAMINATION OF THE RESULTS OF CERTAIN INDIVIDUAL COW TESTS

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INTRODUCTION.

THE work described in this paper was undertaken, primarily, with the object of assessing the seasonal variation of butterfat percentage and of determining the significance, if any, of such variation. An attempt has been made to account for those significant variations which have been found.

It was further considered to be desirable to ascertain the probability of the butterfat percentage of a bulk sample of afternoon or morning milk failing to reach certain specified minimum requirements. These requirements were, first, the presumed legal minimum for genuine milk, and, second, the minimum for "Certified" milk under the Scottish regulations.

With these objects in view, the seasonal means of butterfat percentage of afternoon and of morning milk, together with the respective standard deviations, coefficients of variation and standard errors have been determined.

DATA.

The data comprise some three thousand two hundred results of butterfat determinations, made by the Gerber method, on milk samples from individual cows. The samples were drawn by the Milk Recorder on the occasions of his routine visits to the herd during a period of about five years. The herd numbered between forty and fifty cows.

TREATMENT OF DATA.

It was decided to group the results according to the quarter of the milk recording year during which they were obtained.

The reasons for this treatment were, first, the somewhat uneven intervals between sampling dates—an average of six weeks with a range of from four to nine weeks—and, second, the fact that the number of visits in certain months, notably August, was small. In Table I the "mean fat %" is the mean of all the fat percentages concerned. It is not weighted for the milk yield corresponding to each sample. Weighted means would have been slightly lower than those given. It would appear, however, from certain work on the

correlation of milk yield and butterfat percentage, that the error thus introduced is small. Gaines and Davidson(1) give the correlation coefficient for Holsteins as $r = -0{\cdot}198 \pm 0{\cdot}012$ on the basis of the whole lactation. The average coefficient of correlation for Shorthorns at a single test has been found by the author (unpublished) to be $r = -0{\cdot}153 \pm 0{\cdot}019$.

The seasonal means of butterfat percentage, together with other relevant statistical data, are shown in Table I.

Table I.

Season	No. of tests	Mean fat %	S.D. of single determination	% coefficient of variation	S.E. of mean
		Afternoon samples			
Oct.–Dec.	390	3·59	0·66	18·3	0·033
Jan.–Mar.	500	3·49	0·55	15·6	0·024
Apr.–June	497	3·55	0·59	16·5	0·026
July–Sept.	260	3·63	0·67	18·6	0·042
		Morning samples			
Oct.–Dec.	395	3·12	0·61	19·4	0·031
Jan.–Mar.	491	3·03	0·41	13·6	0·019
Apr.–June	491	2·99	0·53	17·8	0·024
July–Sept.	260	3·13	0·63	20·2	0·039

The different numbers of samples in the morning and afternoon groups in certain seasons are accounted for by souring or spillage.

Discussion.

A. *Statistical reliability of the sample.*

A consideration of the standard errors set out in Table I shows the reliability which can be attributed to the calculated means.

The degree of dispersion, as shown by the percentage coefficient of variation, is relatively small by comparison with those found for certain other biological investigations.

On the more practical side, it may be pointed out that the relatively large number of cows involved, the continuity of management and the period over which the samples were taken, tend to smooth out irregularities due to (*a*) individuality of the cows, (*b*) age, (*c*) period of lactation, and (*d*) sexual excitement. Breed, or at least type, was a constant factor, while abnormal conditions such as may influence results obtained in a show-yard are unlikely to affect results obtained on the farm. Finally, day-to-day variations, in so far as they affect results obtained at intervals of about six weeks as compared with those obtained daily throughout the lactation, have been shown by workers at the National Institute for Research in Dairying, Reading(2) to introduce an error of less than 0·2 per cent. fat.

B. *Seasonal variations.*

(*a*) *Of the mean.*

From Table I it will be seen that the minimum mean seasonal butterfat percentage occurs during the period January–March for afternoon samples

and during April–June for samples of morning milk. The significance which is attached to the seasonal variations is shown in Table II.

Table II.

Season	Difference (S.E. of difference)	Significance
	Afternoon samples	
July–Sept. > Oct.–Dec.	0·79	Not significant
July–Sept. > Apr.–June	1·59	Not significant
July–Sept. > Jan.–Mar.	2·80	Significant $p<0·01$
Oct.–Dec. > Apr.–June	0·86	Not significant
Oct.–Dec. > Jan.–Mar.	2·31	Significant $p<0·05$
Apr.–June > Jan.–Mar.	1·52	Not significant
	Morning samples	
July–Sept. > Oct.–Dec.	0·23	Not significant
July–Sept. > Jan.–Mar.	2·34	Significant $p<0·05$
July–Sept. > Apr.–June	3·08	Significant $p<0·01$
Oct.–Dec. > Jan.–Mar.	2·56	Significant $p<0·05$
Oct.–Dec. > Apr.–June	3·55	Significant $p<0·01$
Jan.–Mar. > Apr.–June	1·28	Not significant

It will be seen that the afternoon means for July–September and October–December are significantly higher than that for January–March. They are not, however, significantly higher than that for April–June, nor is this latter significantly higher than the mean for January–March.

In the case of morning samples, the means during the period July–September and October–December are significantly higher than those for the other two periods. However, the mean for January–March is not significantly higher than that for April–June.

(*b*) *Of the standard deviation.*

It will be seen from Table I that there is a noticeable variation of the standard deviation from season to season in both the afternoon and morning. The standard deviations, together with their respective standard errors, are shown in Table III.

Table III.

Season	S.D.	S.E. of S.D.
	Afternoon samples	
Oct.–Dec.	0·66	0·023
Jan.–Mar.	0·55	0·017
Apr.–June	0·59	0·019
July–Sept.	0·67	0·030
	Morning samples	
Oct.–Dec.	0·61	0·022
Jan.–Mar.	0·41	0·013
Apr.–June	0·53	0·017
July–Sept.	0·63	0·028

In each case the value falls from October–December to January–March and subsequently rises through April–June to reach a maximum in July–September. The significance of these variations has been calculated and is shown in Table IV.

Table IV.

Season	Difference (S.E. of difference)	Significance
	Afternoon samples	
July–Sept. > Oct.–Dec.	0·52	Not significant
July–Sept. > Apr.–June	2·53	Significant $p<0·05$
July–Sept. > Jan.–Mar.	3·76	Significant $p<0·01$
Oct.–Dec. > Apr.–June	2·30	Significant $p<0·05$
Oct.–Dec. > Jan.–Mar.	3·75	Significant $p<0·01$
Apr.–June > Jan.–Mar.	1·58	Not significant
	Morning samples	
July–Sept. > Oct.–Dec.	0·76	Not significant
July–Sept. > Apr.–June	3·09	Significant $p<0·01$
July–Sept. > Jan.–Mar.	7·17	Significant $p<0·01$
Oct.–Dec. > Apr.–June	2·69	Significant $p<0·01$
Oct.–Dec. > Jan.–Mar.	7·66	Significant $p<0·01$
Apr.–June > Jan.–Mar.	5·57	Significant $p<0·01$

A consideration of Table IV (afternoon samples) shows that the standard deviation during the periods July–September and October–December is significantly greater than during April–June or January–March. That for April–June is not, however, significantly greater than that for January–March.

In the morning the standard deviation is significantly greater for the periods July–September and October–December than for the other two periods. Further, the figure for April–June is significantly greater than that for January–March.

While physiological causes may be at the root of these differences it is suggested that the smaller variations during January–March in both afternoon and morning samples may, in part, be accounted for by the uniform conditions of housing and feeding throughout this period. Divergent results between afternoon and morning in the case of period April–June would be expected if the cows were at grass during the day for almost the whole of the period, but housed at night during the first few weeks.

C. *Comparison of the seasonal means with specified standards.*

(*a*) *The presumed legal minimum for genuine milk* (3·0 *per cent.*).

Reference to Table I shows that in no case would a deduction of three times the standard error reduce the mean to 3·0 per cent. in the case of afternoon milk. Under these circumstances it may be concluded that the mean is unlikely to fall below this standard—the odds being more than 700 to 1 against such an occurrence.

It may be noted, in passing, that individual samples during any period may fail to reach 3·0 per cent., as shown by the standard deviation.

The mean for morning milk during the period April–June is somewhat more likely to fall below 3·0 per cent. than to reach this figure; the chances of failure being almost two out of three.

During the period January–March the chances of such failure are about one in seventeen.

(*b*) *The minimum for "Certified" milk under the Scottish regulations* (3·5 *per cent.*).

The mean for the afternoon milk may fall below the required standard during any period. It is about two chances to one that it will do so during January–March.

In no case is the morning milk likely to qualify as the odds against it doing so are at least 700 to 1.

SUMMARY.

Figures showing the means and the standard deviation of butterfat percentage of milk samples, at the afternoon and morning milkings respectively, during the four quarters of the year are presented.

The probable sources of error due to the nature of the sample are discussed and the seasonal variability of mean and of standard deviation is considered.

The likelihood of the mean of afternoon or of morning milk failing to reach certain specified minima during the respective seasons is calculated.

ACKNOWLEDGMENT.

It is desired to acknowledge the help received from Dr Sanders with respect to the statistical work and the criticism and suggestions of Dr Wishart. In addition thanks are due to the owners of the herd for permission to publish the data. The name of the owners, the breed of cows, and the name of the Recording Society are withheld in compliance with a ruling by the Society.

REFERENCES.

(1) GAINES, W. L. and DAVIDSON, F. A. (1923). *Ill. agric. Exp. Sta. Bull.* No. 245, p. 584.

(2) NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING, READING. *Annual Report*, 1928, p. 42.

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90. INVESTIGATIONS ON THE MILK OF A TYPICAL HERD OF SHORTHORN COWS. II

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(With 2 Charts.)

Introduction.

The following paper deals with the fourth and fifth years of an investigation which was concluded in October 1933. An account of the results of the earlier work appeared in a previous paper(1).

Cows.

In that paper(1) a description was given of the ten dairy Shorthorn cows selected in September 1928 to form a small typical herd. During the year 1931–2 eight of the original ten cows continued in the herd and two home-bred heifers were added to maintain the number. In the course of 1932–3 three of the original ten cows were disposed of and one second-calf cow and one heifer, both home-bred, were added to the group.

In Table I information is given regarding the ages of the cows comprising the typical herd, with dates of calving, milk yields and average fat percentages for the two years under review.

Table I. *Milk yield, fat content and dates of calving for typical herd cows.*

Name of cow	Date of calving	Lactation period	Yield of milk lb.	Days in milk	Fat %
Year commencing October 1, 1931–2					
Doll 2 (born 5. xi. 21)	June 13, 1932	8	6,649½	256	3·85
Flora 1 (born 28. xi. 21)	Nov. 7, 1931	8	14,229½	284	3·44
Pearl (born 17. iii. 23)	June 10, 1932	7	10,826½	287	3·81
Rosebud (born 3. xii. 23)	Jan. 8, 1932	6	10,840¾	263	3·36
Winsome 3 (born 14. x. 24)	Feb. 20, 1932	5	11,316	314	3·54
Lottie 2 (born 2. iii. 25)	May 27, 1932	5	5,183½	252	3·45
A. Peach 2 (born 2. iii. 25)	Mar. 2, 1932	5	8,673½	268	3·81
Firefly 3 (born 24. ix. 25)	Jan. 12, 1932	4	8,123¼	311	3·42
A. Red Rose (born 3. xii. 28)	Oct. 2, 1931	1	8,169	361	4·32
Lottie 4 (born 17. iii. 29)	Dec. 21, 1931	1	7,996	281	3·82
Year commencing October 1, 1932–3					
Doll 2 (born 5. xi. 21)	Sold fat	—	—	—	—
Flora 1 (born 28. xi. 21)	Nov. 28, 1932	9	13,152¼	303	3·32
Pearl (born 17. iii. 23)	Aug. 13, 1933	8	8,215¼	256	3·83
Rosebud (born 3. xii. 23)	Apr. 19, 1933	7	8,512½	260	3·21
Winsome 3 (born 14. x. 24)	Apr. 10, 1933	6	9,995½	318	3·50
Lottie 2 (born 2. iii. 25)	Sold fat	—	—	—	—
A. Peach 2 (born 2. iii. 25)	Apr. 10, 1933	6	6,430	227	3·71
Firefly 3 (born 24. ix. 25)	Sold fat	—	—	—	—
A. Red Rose (born 3. xii. 28)	Nov. 28, 1932	2	8,225½	311	4·08
Lottie 4 (born 17. iii. 29)	Jan. 28, 1933	2	8,247½	283	3·50
Carlet 3 (born 25. ii. 28)	Sept. 25, 1932	2	8,355	281	3·57
Cora 1 (born 4. x. 29)	Sept. 5, 1932	1	7,124½	316	3·48

HEALTH AND MANAGEMENT.

Throughout the two years now under review the health of the herd continued to be highly satisfactory. In this connection it may be well to state that periodic examination of the chlorine content and other examinations failed to show the presence of mastitis in the experimental milk. The milk may therefore be regarded as having been normal throughout the experiment. The system of management outlined in the previous paper was continued. During the winter periods the cows were housed during the night and were out on short pasture during the daytime from 10 a.m. to 12 noon. During the summer period and up to mid-October the cows were at pasture day and night.

HOME-GROWN FOODS.

The periods when the different bulky foods were given are shown in Table II.

The weights of hay and mangels given indoors ranged from 7 to 14 lb. hay and 40 to 60 lb. mangels per head, and the green foods given out of doors were from 20 to 50 lb. kale or cabbage and from 25 to 35 lb. silage, according to the apparent requirements of the cows.

Table II. *Showing bulky foods and periods of use from October* 1, 1931 *to September* 30, 1933.

1931–2			1932–3		
Dates	Foods		Dates	Foods	
Oct. 1 – Oct. 26		Pasture	Oct. 1 – Oct. 28		Pasture
Oct. 27 – Nov. 9	Cabbage	Hay	Oct. 28 – Dec. 29	Kale	Hay
Nov. 17 – Apr. May 1	Mangel	Hay	Dec. 30 – Apr. May 2	Mangel	Hay
		Pasture	July 4 – July 19	Silage	Pasture
			July 20 – Aug. 1	Green lucerne	Pasture
			Aug. 2 – Sept. 14	Silage	Pasture
Sept. 27 – Sept. 30	Kale	Pasture	Sept. 15 – Sept. 30	Kale	Hay

CONCENTRATES.

The concentrates given during 1931–2 and 1932–3 were exactly similar in amount and composition to those given in the preceding three years. During the winter the mixture consisted of 3 parts dried ale grains, 2 parts maize-germ meal or maize-germ cake and 1 part extracted soya-bean meal—all parts by weight. During the summer the mixture comprised equal weights of dried

grains and maize-germ meal or cake, and in the late summer and early autumn a proportion of soya-bean meal was introduced, giving a mixture of 2 parts dried grains, 2 parts maize-germ meal and ½ part soya-bean meal.

The quantities given ranged from 3½ to 3¾ lb. for each gallon of milk per cow daily during the winter to 3 lb. for each gallon of milk over three per day during the best part of the grazing period. In later summer and autumn the amount given was controlled according to the estimated feeding value of the pasture and other bulky foods given. Throughout the two years all cows ate the concentrate mixture readily.

Sampling and testing of milk.

Throughout the two-year period under review, the sampling and testing were carried out in the way described in the previous paper(1), except that from July 29, 1933, the total solids were determined by a new method(2).

Daily records and weekly averages.

It will be seen on reference to Table III that the number of cows contributing milk in any week ending on the date shown in column 1 was never less than six.

The dates during the week on which cows were brought into, or taken out of, the herd are shown in column 2. The figures refer to the number of cows in milk at the end of the week. The introduction of a freshly calved cow was always followed by an increase in the quantity of milk yielded during the week. This is shown in column 3. This increase overcame the reduction in the quantity of milk yielded by the remainder of the cows due to advancing lactation, and the total yield of milk rose for a period of 7–23 days, during which time the increasing yield from the newcomer more than balanced the falling off of the milk from the remainder.

The removal of dry cows had little influence on the quantity of milk for the week, as the fall in milk yield had already been spread over a longer period.

These changes in the composition of the herd are largely responsible for the variations in the quantity of milk given per week. There are, however, two exceptions when the normal fall in yield due to advancing lactation is converted into a rise; in May 1932 and also at the end of April 1933 a slight rise was observed in the milk of five cows. The large rise shown in Table III, column 3, at the end of April and the beginning of May 1933 was chiefly due to the introduction of three freshly calved cows. Such a rise in the quantity of milk as followed the flush of grass in the spring was not observed to result from any other changes in the feeding.

The percentages of butterfat in the evening's and morning's milk for each week were calculated by dividing the total weight of fat × 100 by the weight of milk for the week. The evening's milk was only once below 4 per cent., viz.

Table III. *Daily records and weekly averages.*

Daily evening and morning tests, of composite samples made up from the milk of the individual cows daily. The results are averaged for each week of the two years.

Column 1 shows the date of the *end* of each week to which the figures in the same line refer.

Column 2 shows the date on which changes were made in the number of cows to the number at the end of each week.

Column 3 gives the total weight of milk during the week, evening and morning.

Column 4 gives the average percentage of fat p.m. and a.m.

Column 5 gives the mean of the percentages of solids-not-fat p.m. and a.m.

1	2		3		4		5	
Week ending	No. of cows		Weight of milk lb.		Fat %		Solids-not-fat %	
1931	Changed on	To	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.
Oct. 4		6	499·25	704·25	4·62	3·31	8·93	9·02
„ 11	6th	7	536·25	797·75	4·77	3·43	8·96	9·08
„ 18		7	561·75	801·25	4·82	3·35	8·96	9·10
„ 25		7	550·25	772·00	4·81	3·24	8·91	9·14
Nov. 1		7	540·50	753·50	4·62	3·25	9·04	9·13
„ 8		7	512·50	712·00	4·48	3·23	8·95	9·19
„ 15	11th	8	523·00	769·75	4·79	3·46	8·99	9·16
„ 22		8	594·25	844·00	4·73	3·48	9·02	9·15
„ 29	23rd	7	568·50	806·25	4·71	3·76	8·90	9·03
Dec. 6	30th	6	555·25	801·00	4·64	3·66	8·97	9·12
„ 13		6	554·75	782·50	4·69	3·50	8·92	9·05
„ 20		6	528·75	738·25	4·59	3·35	8·96	9·08
„ 27	25th	7	536·75	774·25	4·51	3·25	8·99	9·07
1932								
Jan. 3		7	546·75	833·00	4·51	3·33	9·07	9·18
„ 10	4th	6	547·25	811·00	4·60	3·47	9·07	9·14
„ 17	12th, 16th	8	630·75	943·50	4·94	3·45	9·05	9·18
„ 24		8	731·50	1082·50	4·74	3·39	9·07	9·15
„ 31		8	725·00	1098·75	4·74	3·37	9·09	9·17
Feb. 7		8	699·75	1015·00	4·79	3·31	9·03	9·09
„ 14	8th	7	677·50	939·50	4·55	3·30	9·05	9·09
„ 21		7	656·75	927·25	4·42	3·25	9·02	9·07
„ 28	25th	8	656·50	962·50	4·49	3·19	8·90	8·96
Mar. 6	3rd	7	716·25	1038·25	4·59	3·32	8·96	8·96
„ 13	7th	8	789·25	1165·75	4·49	3·29	8·95	8·97
„ 20		8	747·50	1157·75	4·41	3·30	8·91	8·89
„ 27	24th	7	733·75	1153·00	4·34	3·33	8·89	8·86
Apr. 3		7	740·25	1121·00	4·47	3·23	8·84	8·85
„ 10		7	714·00	1097·75	4·51	3·26	8·79	8·81
„ 17		7	711·00	1079·75	4·37	3·34	8·79	8·80
„ 24		7	699·50	1049·25	4·43	3·43	8·79	8·81
May 1		7	723·00	1098·50	4·63	3·31	8·88	8·86
„ 8		7	783·25	1206·75	4·50	3·12	8·83	8·94
„ 15		7	796·75	1220·25	4·28	2·97	8·85	8·97
„ 22		7	827·50	1216·25	4·26	2·89	8·79	8·94
„ 29		7	790·25	1113·75	4·34	2·92	8·69	8·86
June 5	2nd	8	770·50	1135·50	4·19	3·03	8·67	8·80
„ 12		8	822·50	1180·50	4·22	2·90	8·65	8·87
„ 19	15th	9	871·00	1317·75	4·33	2·97	8·70	8·92
„ 26		9	933·50	1382·50	4·26	2·98	8·73	8·93
July 3		9	964·75	1367·00	4·33	2·89	8·71	8·90
„ 10	5th	10	974·50	1430·75	4·33	3·02	8·67	8·84
„ 17		10	956·00	1415·75	4·28	3·25	8·57	8·77
„ 24		10	968·00	1389·75	4·26	3·19	8·61	8·79
„ 31		10	933·00	1372·50	4·35	3·07	8·71	8·86
Aug. 7		10	854·50	1309·50	4·34	3·14	8·64	8·77
„ 14		10	805·00	1269·75	4·31	3·16	8·60	8·77
„ 21		10	799·00	1138·25	4·36	3·08	8·61	8·73
„ 28	21st	9	750·00	1122·00	4·60	3·21	8·73	8·83
Sept. 4		9	722·75	1104·25	4·61	3·29	8·76	8·85
„ 11		9	686·50	997·25	4·75	3·37	8·72	8·82
„ 18		9	662·25	932·00	4·78	3·43	8·73	8·90
„ 25		9	587·75	856·50	4·88	3·62	8·78	8·89

Table III (*continued*).

1 Week ending 1932	2 No. of cows		3 Weight of milk lb.		4 Fat %		5 Solids-not-fat %	
	Changed on	To	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.
Oct. 2	27th, 29th	11	677·00	987·25	4·74	3·68	8·75	8·93
„ 9		11	792·75	1129·75	4·69	3·50	8·83	8·92
„ 16		11	765·25	1086·50	4·39	3·36	8·80	8·93
„ 23		11	737·75	1037·00	4·37	3·32	8·72	8·76
„ 30	27th	10	662·50	891·25	4·44	3·43	8·77	8·78
Nov. 6		10	613·50	836·50	4·33	3·48	8·67	8·71
„ 13	12th	9	551·00	797·75	4·28	3·52	8·64	8·64
„ 20		9	513·25	748·75	4·53	3·35	8·73	8·67
„ 27		9	484·75	704·25	4·30	3·22	8·67	8·63
Dec. 4	*	10	498·00	750·75	4·26	3·30	8·73	8·82
„ 11		10	633·00	948·25	4·54	3·62	8·87	8·98
„ 18		10	654·00	1001·00	4·41	3·47	8·79	8·86
„ 25		10	667·25	1008·25	4·38	3·36	8·79	8·78
1933								
Jan. 1		10	601·75	1012·25	4·49	3·27	8·73	8·71
„ 8	2nd, 3rd, 4th	7	562·25	865·50	4·27	3·29	8·85	8·85
„ 15		6	560·75	833·50	4·30	3·35	8·84	8·87
„ 22		6	548·50	797·25	4·49	3·36	8·80	8·77
„ 29		6	510·50	801·50	4·26	3·33	8·94	8·85
Feb. 5	1st	7	544·25	844·25	4·32	3·25	8·89	8·83
„ 12		7	596·50	929·50	4·08	3·08	8·89	8·89
„ 19		7	580·75	903·75	4·23	3·11	8·99	8·90
„ 26		7	543·50	873·50	4·29	3·26	9·01	8·84
Mar. 5	2nd	6	546·00	841·50	4·09	3·10	9·03	8·90
„ 12		6	540·50	826·00	4·16	3·18	8·94	8·88
„ 19		6	525·25	811·00	4·17	3·13	9·00	8·97
„ 26		6	503·75	777·25	4·27	3·17	9·03	8·97
Apr. 2		6	491·00	753·00	4·21	3·08	8·97	8·88
„ 9		6	469·75	717·50	4·34	3·14	8·93	8·89
„ 16		6	453·25	731·00	4·28	3·15	8·97	8·85
„ 23	†	6	528·50	868·50	4·36	3·10	9·03	8·94
„ 30	23rd, 26th	8	771·00	1212·00	4·36	3·11	9·09	8·98
May 7		8	922·00	1348·25	4·25	2·86	8·97	9·01
„ 14		8	810·25	1365·50	4·23	2·79	8·97	9·04
„ 21		8	950·50	1348·50	4·11	2·62	8·82	8·99
„ 28		8	947·75	1312·75	4·22	2·77	8·73	8·89
June 4		8	922·25	1291·75	4·17	2·78	8·72	8·91
„ 11		8	847·00	1222·75	4·09	2·94	8·54	8·80
„ 18		8	809·50	1160·25	4·26	2·94	8·55	8·83
„ 25		8	751·00	1095·75	4·40	3·08	8·56	8·82
July 2		8	704·50	1060·25	4·39	3·11	8·49	8·73
„ 9		8	641·25	969·25	4·40	3·17	8·34	8·58
„ 16	10th	7	626·75	937·75	4·47	3·01	8·51	8·69
„ 23		7	588·00	899·50	4·27	3·18	8·36	8·58
„ 30		7	542·00	872·00	4·19	3·28	8·26	8·56
Aug. 6		7	518·25	814·25	4·16	3·18	8·24	8·44
„ 13		7	464·25	771·25	4·04	3·25	8·06	8·33
„ 20	‡	7	498·75	787·00	4·03	3·22	8·18	8·40
„ 27		7	532·75	889·75	3·93	3·40	8·25	8·49
Sept. 3		7	530·50	879·00	4·07	3·29	8·27	8·44
„ 10		7	547·25	859·00	4·20	3·22	8·35	8·49
„ 17	15th	8	535·00	856·25	4·16	3·24	8·36	8·57
„ 24	18th	7	602·25	902·00	4·17	3·00	8·49	8·63
Oct. 1		7	604·00	890·25	4·39	3·08	8·44	8·54
„ 8	5th	9	701·50	1055·00	4·43	3·18	8·58	8·66
„ 15		9	872·00	1222·25	4·37	3·13	8·60	8·62

* One cow dried off on December 1, two cows brought in on December 2.

† One cow brought in on April 19, one cow discontinued on April 22.

‡ One cow dried off on August 16, one cow brought in on August 17. June 2, 1932: Lottie 2 milk from 2 quarters only—other two abnormal. July 5, 1932: Doll 2 milk from 3 quarters included—fourth quarter abnormal.

on the week ending August 27, 1933 (see Table III, column 4). The daily percentage of fat in the mixed evening's milk on any day was only once as low as 3·62, viz. on August 16, 1933.

The average percentage of fat in the morning's milk (with one exception) was below 3 from the week ending on May 15 to July 10, 1932, and again from the week ending on May 7 to June 25, 1933. Column 5, Table III, gives the average percentage of solids-not-fat for the week ending on the date shown in column 1. This figure is the mean of the seven daily results p.m. and a.m.

The seasonal variations in the solids-not-fat during the year 1933 presented a picture quite different from any of the four preceding years, in which there had been no serious drought. Up to the week ending June 11, 1933, the solids-not-fat had been fairly high, but during that week the percentage of this constituent in the evening's milk began to fall and thereafter low figures were obtained each week till October 1933.

DAILY RESULTS DURING DROUGHT PERIOD 1933.

During the summer of 1933 the milk of the typical herd differed so much from that of the four previous years that it is well to consider the results of the daily analyses in detail.

The factor which was responsible for the differences noted was the drought extending from July 16 to September 12. The rainfall in inches was as follows:

Mar.	Apr.	May	June	July	*Aug.*	Sept.	Oct.
2·5	1·23	1·96	1·17	1·3	*0·38*	2·76	1·5

In 1929 and 1930 it had been necessary to supplement the grazing, and some indication had been obtained of a fall in solids-not-fat under these conditions. In 1933 supplementary feeding was necessary and the milk differed in composition from previous years to a greater extent.

The exact causes of this change, characterised by a fall in the percentage of solids-not-fat, have not yet been discovered, but they are associated with drought conditions.

Chart 1 shows the daily variation both at night and in the morning in the weight of milk, and the percentages of fat and of solids-not-fat which it contained from May 1 to September 10, 1933.

WEIGHT OF MILK.

The inclusion of the milk of three recently calved cows is mainly responsible for the high yield of milk during May, although the falling milk yield of the remaining five cows was arrested and turned into a gain on the early spring grass. From July 9 onwards the normal fall in milk yield caused by advancing lactation was resumed for all cows, but there was no abnormal drop in quantity

Daily yield and composition of milk during the summer of 1933.

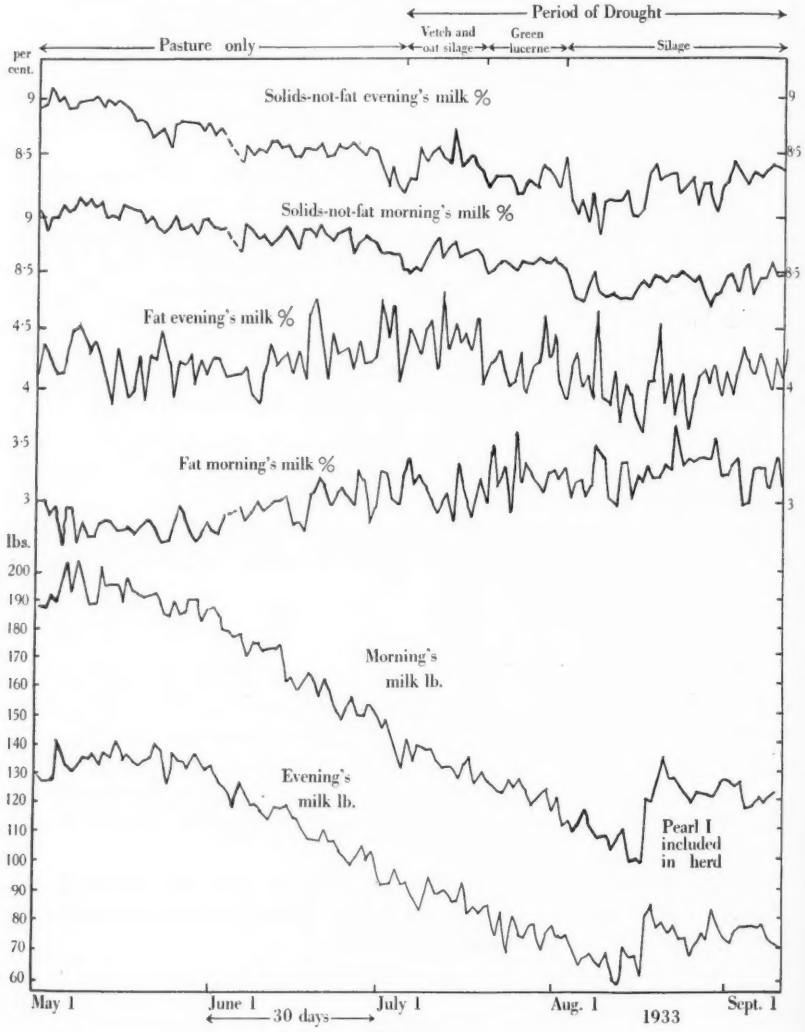

Chart 1.

of milk yielded. The introduction of Pearl 1, a freshly calved cow, raised the yield on August 17.

FAT.

The percentage of fat in the morning's milk fell below 3 from May 2 to June 8; similar results had been noted in the four previous years when the cows were on fresh spring pasture.

The butterfat in the morning's milk again fell below 3 per cent. fat on thirteen occasions between June 8 and July 16. During the succeeding period of drought the percentage of fat in the morning's milk was distinctly higher, rising on three occasions above 3·5. This may have been partly accounted for by the fact that the percentage of fat in the evening's milk fell during this period and was below 4 on 16 days (see Chart 1).

During May and June the percentage of fat in the evening's milk with only seven exceptions remained above 4; the chief cause for the fat in the morning's milk being below the minimum presumptive standard is associated with the varying intervals of time between milkings.

SOLIDS-NOT-FAT.

The solids-not-fat which, with the exception of the short period in the summer of 1930, had remained in the neighbourhood of 9 per cent., fell, as the summer advanced and during the period of drought to below 8·5 per cent. The evening's milk was below 8 per cent. on four occasions. The feeding of vetch and green lucerne seemed to improve this condition slightly, although the night's milk was still below 8·5. During August both the night's and the morning's milk was below the presumptive standard (see Chart 1).

Calculations showed that the solids in the *fat-free milk* were below 8·5 per cent. in the night's milk on twelve occasions, showing that the higher fat was not responsible for the low non-fatty solids.

COMPOSITE SAMPLE OF TYPE HERD—WEEKLY ANALYSES OF MORNING MILK, OCTOBER 6, 1931–SEPTEMBER 26, 1933.

In addition to the analyses made weekly during the first three years of this experiment, determinations of the *p*H and of the freezing point were made as frequently as possible between October 1931 and September 1933. The titratable acidity, the formol titration, the rennet test, the estimations of ash, calcium and phosphorus, were carried out by the methods already detailed (1). The Hortvet (3) method for freezing point depression was substituted for that of Andrews on February 2, 1932, and was used in all subsequent determinations. The *p*H was measured with a Cambridge Instrument Co.'s Potentiometer, using quinhydrone and gold half-electrodes (Biilman (4)); values were corrected for temperature to 18° C. The results are shown in Tables IV and V and graphically in Chart 2, A–K. Results obtained on

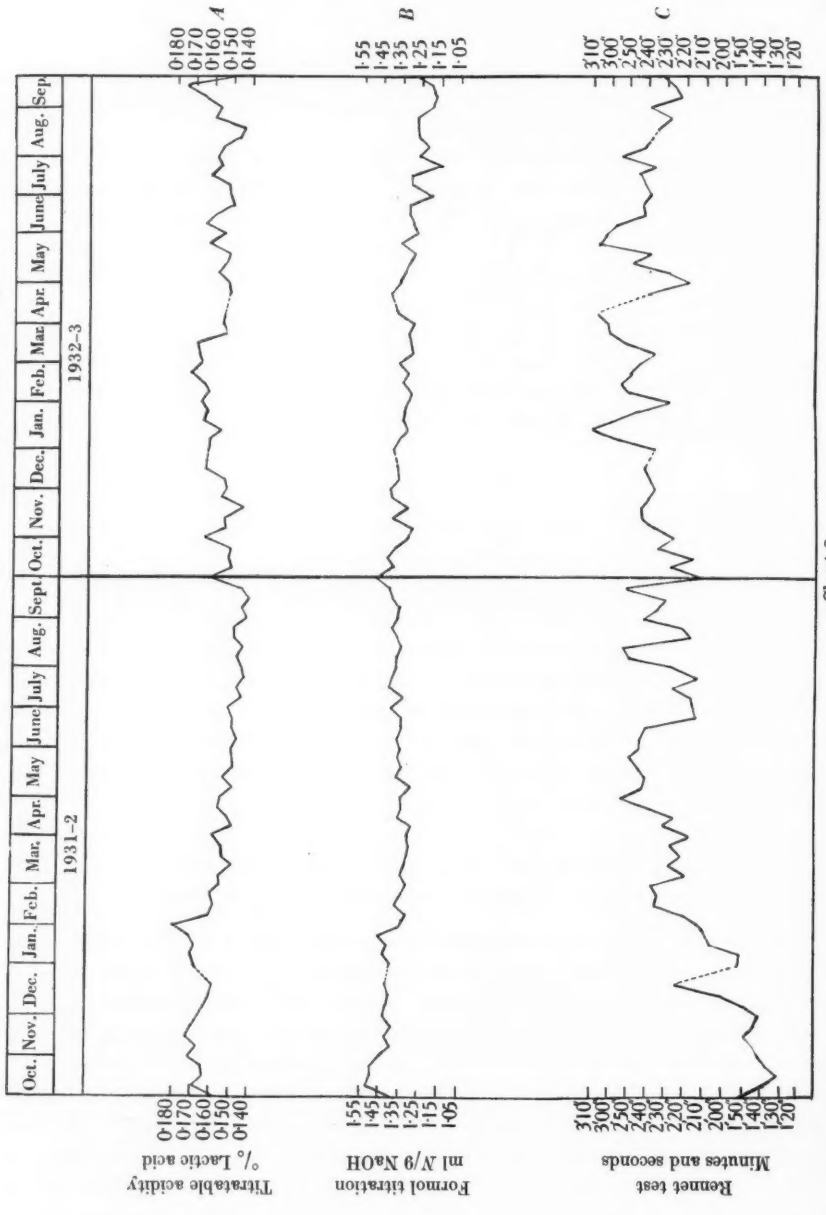
Titratable acidity, formol titration and rennet test of weekly samples.
Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept.
1931–2
1932–3
0·180 0·170 0·160 0·150 0·140
A
1·55 1·45 1·35 1·25 1·15 1·05
B
3'10" 3'00" 2'50" 2'40" 2'30" 2'20" 2'10" 2'00" 1'50" 1'40" 1'30" 1'20"
C
Titratable acidity % Lactic acid
Formol titration ml N/9 NaOH
Rennet test Minutes and seconds

Chart 2.

Chart 2 (*continued*).

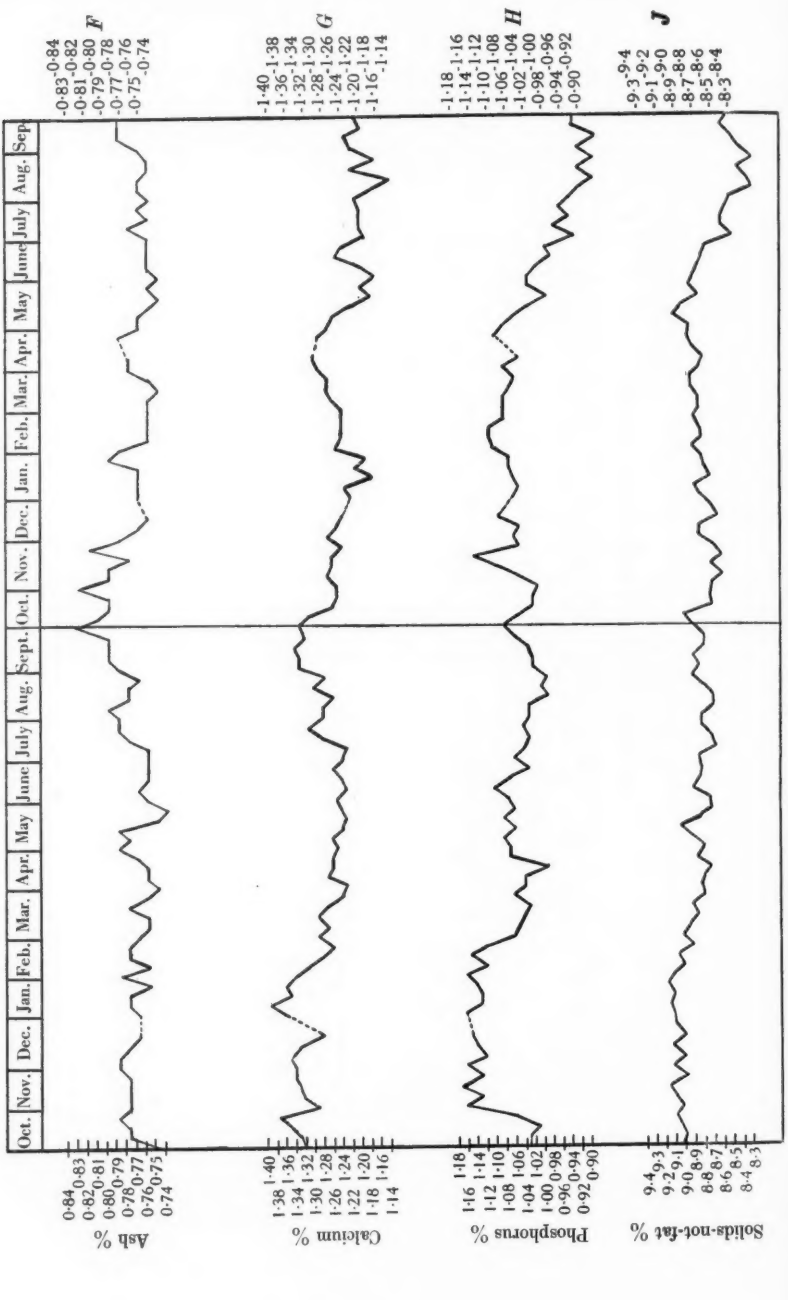
Ash, calcium, phosphorus and solids-not-fat in weekly samples
Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Aug. Sept.
Ash %
Calcium %
Phosphorus %
Solids-not-fat %
F
G
H
J

Chart 2 (*continued*).

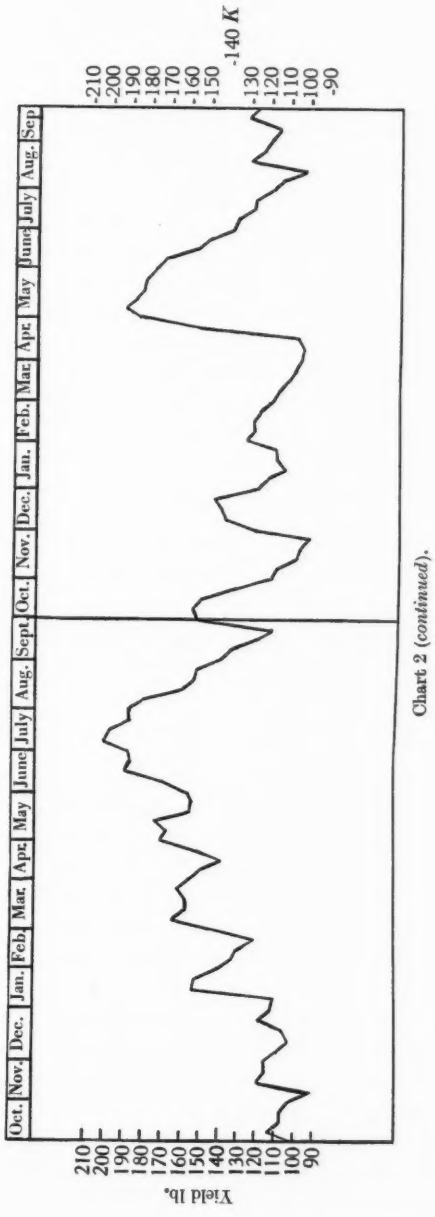

Chart 2 (*continued*).

October 3, 10, 17, 1933, are given at the end of Tables IV and V although not strictly within the dates of experimental period.

For comparison with the results of the earlier years, the highest and lowest values for the period 1931–3 may be considered.

Titratable acidity.

The highest and lowest values of the titratable acidity for the period October 1931 to September 1932 were 0·18 and 0·14; for 1932–3 the corresponding values were 0·17 and 0·14, the higher values being obtained during the months October to February, when the total yield of milk from the herd is lowest; this is rather unexpected, since, as pointed out in the first section, the total yield was always increased after the introduction of a freshly calved cow, with milk of a higher acidity than those in late lactation. The general trend of the acidity variations agrees with that of the previous three periods, and it was therefore concluded that the acidity was slightly lowered when the cows were on pasture (Table IV and Chart 2 A).

Formol titration.

The highest and lowest values of 1·51 and 1·27 for 1931–2 and 1·47 and 1·15 for 1932–3 are lower than have hitherto been found. As in the case of the titratable acidity, the higher values occurred during the months October to February. After the beginning of July 1933, the values for this period were generally lower than usual (Table IV and Chart 2 B).

Rennet test.

During 1931–2 the longest time of coagulation with rennet was 2 min. 53 sec. and the shortest 1 min. 32 sec. During 1932–3 the longest time taken was 3 min. 9 sec. and the shortest 2 min. 12 sec. Except in one or two isolated cases the more rapid coagulation was found to occur during October, November and December 1931, becoming rather variable but generally slower until May and June; during these months the test was never complete in less than 2 min. 40 sec. During July 1931 when the yield of milk was greatest the results were more rapid.

That the introduction of freshly calved cows has a material influence upon the rennet test of the composite sample was demonstrated by a test on October 10, 1933 (a fortnight after the completion of main experiment. See figures at end of Tables IV and V). With a titratable acidity increased only by 0·02 from that of the previous week, the time of coagulation with rennet was found to have decreased by 23 sec. The cause of this sudden quickening of the test was found to be the introduction of the milk of two freshly calved cows; with acidities of 0·205 and 0·195 this milk contributed more than half of the bulk sample.

Table IV. *Weekly determinations.*

Date 1931	Titratable acidity (*N*/9 NaOH)	Formol titration (*N*/9 NaOH)	*p*H	Rennet test min.	Rennet test sec.	Freezing-point ° C.
Oct. 6	0·16	1·37	—	1	52	−0·551
„ 13	0·17	1·51	—	1	38	−0·556
„ 20	0·16	1·49	—	1	32	−0·559
„ 27	0·16	1·49	—	1	37	−0·556
Nov. 3	0·17	1·44	—	1	41	−0·563
„ 10	0·17	1·39	—	1	44	−0·559
„ 17	0·17	1·41	—	1	48	−0·562
„ 24	0·17	1·39	—	1	44	−0·572
Dec. 1	0·17	1·42	6·67	1	41	−0·561
„ 8	0·16	1·41	—	1	48	−0·561
„ 15	0·16	1·40	6·69	2	1	−0·570
„ 22	0·16	1·41	6·90	2	24	−0·567
1932						
Jan. 5	0·17	1·39	6·76	1	52	−0·567
„ 12	0·17	1·42	6·75	1	51	−0·562
„ 19	0·17	1·41	6·70	2	6	−0·564
„ 26	0·17	1·45	6·75	2	8	−0·566
Feb. 2	0·18	1·33	6·73	2	11	−0·553
„ 9	0·16	1·31	6·88	2	19	−0·547
„ 16	0·16	1·36	6·75	2	35	−0·546
„ 23	0·16	1·33	—	2	33	−0·547
Mar. 1	0·16	1·31	6·83	2	37	−0·542
„ 8	0·16	1·34	6·76	2	19	−0·549
„ 15	0·15	1·27	6·82	2	27	−0·546
„ 22	0·15	1·31	6·88	2	22	−0·547
„ 29	0·15	1·30	6·98	2	26	−0·547
Apr. 5	0·16	1·31	6·78	2	17	−0·552
„ 12	0·15	1·29	6·65	2	30	−0·546
„ 19	0·15	1·35	6·75	2	25	−0·546
„ 26	0·16	1·34	6·77	2	38	−0·554
May 3	0·16	1·34	6·74	2	53	−0·554
„ 10	0·15	1·29	6·78	2	42	−0·550
„ 17	0·15	1·37	6·81	2	40	−0·546
„ 24	0·15	1·34	6·98	2	44	−0·549
„ 31	0·15	1·36	6·69	2	48	−0·548
June 7	0·15	1·35	6·70	2	43	−0·548
„ 14	0·15	1·36	6·91	2	43	−0·549
„ 21	0·15	1·34	6·64	2	40	−0·547
„ 28	0·15	1·34	6·76	2	14	−0·548
July 5	0·15	1·39	6·74	2	15	−0·550
„ 12	0·14	1·33	6·83	2	16	−0·548
„ 19	0·15	1·40	6·90	2	26	—
„ 26	0·14	1·39	6·75	2	13	—
Aug. 2	0·14	1·37	—	2	26	−0·547
„ 9	0·15	1·37	—	2	48	−0·548
„ 16	0·14	1·35	—	2	53	−0·547
„ 23	0·15	1·36	—	2	17	−0·549
„ 30	0·15	1·39	6·75	2	22	−0·545
Sept. 6	0·14	1·37	6·72	2	42	−0·546
„ 13	0·14	1·36	6·81	2	36	−0·551
„ 20	0·14	1·39	6·85	2	20	—
„ 27	0·14	1·40	6·74	2	50	−0·546
Oct. 4	0·16	1·47	6·76	2	12	−0·552
„ 11	0·15	1·39	6·57	2	27	−0·549
„ 18	0·15	1·41	6·72	2	16	−0·543
„ 25	0·15	1·38	6·69	2	34	−0·547
Nov. 1	0·16	1·31	6·64	2	26	−0·544
„ 8	0·15	1·29	6·81	2	36	−0·550
„ 15	0·15	1·39	6·81	2	42	−0·544
„ 22	0·14	1·31	6·69	2	43	−0·546

Table IV (*continued*).

Date 1932	Titratable acidity (*N*/9 NaOH)	Formol titration (*N*/9 NaOH)	*p*H	Rennet test min.	Rennet test sec.	Freezing-point ° C.
Nov. 29	0·16	1·39	6·74	2	39	−0·548
Dec. 6	0·15	1·40	6·74	2	37	−0·549
„ 13	0·15	1·36	6·81	2	39	−0·550
„ 20	0·16	1·36	6·76	2	42	−0·548
1933						
Jan. 3	0·16	1·38	6·76	2	37	−0·547
„ 10	0·16	1·36	6·64	2	55	−0·546
„ 17	0·16	1·32	6·67	3	9	−0·554
„ 24	0·17	1·34	6·71	2	59	−0·548
„ 31	0·16	1·33	6·71	2	45	−0·551
Feb. 7	0·17	1·31	6·85	2	29	−0·546
„ 14	0·16	1·30	6·72	2	49	−0·545
„ 21	0·17	1·34	6·78	2	53	−0·548
„ 28	0·17	1·32	—	2	48	−0·547
Mar. 7	0·17	1·36	6·65	2	43	−0·548
„ 14	0·17	1·29	6·68	2	36	−0·548
„ 21	0·17	1·30	6·71	2	51	—
„ 28	0·15	1·31	6·72	3	0	−0·554
Apr. 4	0·16	1·28	6·73	3	1	−0·545
„ 11	0·15	1·37	6·73	3	6	−0·556
„ 18	—	—	—	—		—
„ 25	0·15	1·40	—	2	38	−0·562
May 2	0·15	1·36	—	2	19	−0·559
„ 9	0·16	1·33	—	2	29	−0·559
„ 16	0·15	1·31	—	2	47	−0·559
„ 23	0·15	1·29	6·64	2	39	−0·541
„ 30	0·16	1·35	6·71	3	6	−0·550
June 6	0·15	1·27	6·71	3	2	−0·546
„ 13	0·16	1·29	6·57	2	57	−0·544
„ 20	0·16	1·31	6·74	2	42	−0·541
„ 27	0·15	1·31	6·77	2	43	−0·541
July 4	0·15	1·20	6·74	2	38	−0·541
„ 11	0·15	1·30	6·76	2	42	−0·542
„ 18	0·16	1·30	6·76	2	44	−0·543
„ 25	0·16	1·15	7·00	2	37	—
Aug. 1	0·16	1·26	—	2	54	—
„ 8	0·16	1·22	—	2	42	−0·545
„ 15	0·15	1·26	—	—		−0·547
„ 22	0·14	1·27	—	2	35	—
„ 29	0·16	1·27	—	2	28	−0·541
Sept. 5	0·16	1·20	—	2	39	−0·537
„ 12	0·17	1·18	—	2	23	−0·540
„ 19	0·17	1·20	—	2	26	−0·544
„ 26	0·15	1·28	—	2	32	−0·541
Oct. 3	0·15	1·22	—	2	34	−0·550
„ 10	0·17	1·48	6·33	2	11	−0·550
„ 17	0·17	1·36	—	2	6	−0·542

There appears, however, to be no definite correlation between the titratable acidity and the rennet test of these composite samples, but a negative correlation value with the total calcium content is indicated by statistical calculations. It is intended to deal with this aspect of our findings in a later paper.

The very definite observation which was made during the first three periods of the experiment that the time of coagulation in winter was much longer than in summer was not borne out in these two later periods (Table IV and Chart 2 C).

*p*H DETERMINATION.

The value for the true acidity as determined with the quinhydrone and gold half-electrodes during the periods 1931–2 and 1932–3 lay between 6·98 and 6·64 and 6·85 and 6·57 respectively. The small variations do not appear to be seasonal nor to be correlated with the time of coagulation with rennet as was thought possible, but insufficient determinations have as yet been made, and all variations are so slight that it is not possible to draw any very definite conclusion as to the relative importance of the determination of the hydrogen-ion concentration in mixed samples of this type (Table IV and Chart 2 D).

FREEZING-POINT.

After the introduction of the Hortvet apparatus (on February 2, 1931) for the freezing-point determination, very constant results were obtained, no values lower than −0·554 or higher than −0·542 being found between this date and September 27, 1931. From October 4, 1932, until the end of the experiment on September 26, 1933, the values were rather more variable but were always between −0·562 and −0·537, each of these extreme values occurring only once (Table IV and Chart 2 E).

ASH.

The highest and lowest values for 1931–2 and 1932–3 were 0·80 and 0·74 and 0·83 and 0·75 per cent. respectively. The higher values were definitely found to occur during September, October and November. This seasonal difference was apparently not so noticeable during the period 1928–31 but is clearly seen on reference to Chart 2 F. It is noticeable that between the dates December 20, 1932, and September 12, 1933, the value of 0·77 was only exceeded on six occasions out of thirty-eight, but during the same period in the previous year this value was exceeded on seventeen occasions. The significance of this fact and its relation to the low solids-not-fat during the summer of 1933 can only be elucidated after further investigation and statistical analysis (Table V and Chart 2 F).

CALCIUM.

The seasonal variation in the percentage of calcium in the mixed milk of the experimental herd is again apparent in the figures for 1931–2, the higher values occurring during October–February with consistently lower ones during the summer period until August, rising slightly in September and October; but during the 1932–3 period the higher values of 1931–2 were never reached. The highest value for 1931–2 was 0·140 and the lowest 0·124 per cent., but for 1932–3 the highest value was 0·132 and the lowest 0·115 per cent. (Table V and Chart 2 G).

Table V. *Weekly analyses.*

Date 1931	Yield in lb.	Fat %	Solids-not-fat %	Ash %	Calcium %	Phosphorus %
Oct. 6	103	3·32	9·08	0·75	0·133	0·104
„ 13	116	3·45	9·03	0·78	0·134	0·104
„ 20	109¾	3·45	9·09	0·78	0·136	0·102
„ 27	110½	3·25	9·12	0·79	0·138	0·107
Nov. 3	106½	3·18	9·08	0·78	0·130	0·117
„ 10	94½	3·32	9·12	0·78	0·133	0·114
„ 17	121¾	3·42	9·20	0·78	0·134	0·118
„ 24	118	3·65	9·01	0·78	0·135	0·114
Dec. 1	118	3·55	9·15	0·79	0·135	0·117
„ 8	112	3·60	9·04	0·79	0·136	0·113
„ 15	106¾	3·58	9·16	0·78	0·134	0·115
„ 22	109	3·32	9·04	0·77	0·129	0·116
„ 29	121¾	3·12	9·12	—	—	—
1932						
Jan. 5	115	3·35	9·13	0·77	0·137	0·117
„ 12	113¾	3·55	9·18	0·78	0·140	0·114
„ 19	157¾	3·42	9·15	0·78	0·136	0·114
„ 26	156¾	3·42	9·20	0·76	0·137	0·115
Feb. 2	143½	3·28	9·14	0·79	0·135	0·117
„ 9	136	3·30	9·03	0·76	0·132	0·113
„ 16	134	3·42	9·10	0·78	0·129	0·116
„ 23	124½	3·18	8·96	0·78	0·127	0·113
Mar. 1	148¾	3·35	9·05	0·77	0·130	0·107
„ 8	167¼	3·00	8·99	0·76	0·128	0·106
„ 15	161¼	3·30	8·90	0·76	0·130	0·105
„ 22	162	3·32	8·93	0·78	0·128	0·104
„ 29	165¾	3·22	8·82	0·76	0·125	0·107
Apr. 5	159	3·28	8·86	0·75	0·124	0·105
„ 12	154	3·48	8·82	0·76	0·128	0·105
„ 19	143	3·55	8·77	0·76	0·127	0·100
„ 26	155¾	3·08	8·90	0·77	0·127	0·108
May 3	174¾	3·10	8·84	0·79	0·126	0·108
„ 10	172¼	3·10	8·94	0·78	0·127	0·109
„ 17	177¾	2·65	9·05	0·79	0·125	0·107
„ 24	159½	2·72	8·87	0·75	0·124	0·109
„ 31	158½	3·00	8·76	0·74	0·125	0·107
June 7	160¼	2·88	8·78	0·76	0·126	0·108
„ 14	174½	2·88	8·92	0·77	0·124	0·111
„ 21	193¾	2·88	8·88	0·76	0·125	—
„ 28	191¾	2·88	8·86	0·76	0·127	0·104
July 5	192	2·98	8·89	0·76	0·125	0·107
„ 12	204¾	3·50	8·70	0·76	0·124	0·105
„ 19	201¾	3·25	8·73	0·78	0·129	0·104
„ 26	191¾	3·02	8·84	0·79	0·132	0·105
Aug. 2	192¾	2·80	8·85	0·79	0·129	0·104
„ 9	184	2·90	8·74	0·80	0·129	0·104
„ 16	163	3·22	8·74	0·78	0·127	0·100
„ 23	157¼	3·38	8·82	0·78	0·131	0·101
„ 30	156¾	3·15	8·93	0·77	0·129	0·100
Sept. 6	143½	3·38	8·89	0·79	0·134	0·103
„ 13	138½	3·52	8·92	0·80	0·134	0·103
„ 20	125½	3·40	8·84	0·80	0·135	0·104
„ 27	117	3·50	8·82	0·80	0·133	0·107
Oct. 4	157¼	3·78	8·92	0·82	0·134	0·109
„ 11	158	3·40	9·01	0·81	0·132	0·106
„ 18	153½	3·22	8·74	0·80	0·127	0·103
„ 25	136½	3·62	8·76	0·80	0·126	0·103
Nov. 1	116¾	3·35	8·75	0·83	0·126	0·102
„ 8	113½	3·32	8·64	0·80	0·128	0·106
„ 15	104¾	3·25	8·74	0·80	0·127	0·110
„ 22	102¼	3·20	8·64	0·78	0·127	0·115

Table V (*continued*).

Date 1932	Yield in lb.	Fat %	Solids-not-fat %	Ash %	Calcium %	Phosphorus %
Nov. 29	97½	3·25	8·71	0·82	0·125	0·106
Dec. 6	126¼	3·60	8·86	0·79	0·128	0·107
„ 13	141½	3·48	8·87	0·77	0·126	0·106
„ 20	143¼	3·35	8·69	0·76	0·125	0·110
„ 27	147¾	3·18	8·72	—	—	—
1933						
Jan. 3	124½	3·15	8·87	0·77	0·123	0·107
„ 10	119¾	3·25	8·91	0·77	0·124	0·106
„ 17	110½	3·38	8·75	0·77	0·119	0·107
„ 24	114	3·33	8·81	0·77	0·122	0·108
„ 31	115	3·46	8·82	0·80	0·120	0·108
Feb. 7	130½	3·22	8·92	0·79	0·126	0·111
„ 14	127¼	3·18	8·86	0·76	0·125	0·112
„ 21	127	3·05	8·88	0·76	0·125	0·112
„ 28	123½	3·05	8·91	0·76	0·125	0·109
Mar. 7	116¼	3·25	8·89	0·76	0·125	0·109
„ 14	113¾	3·02	8·89	0·76	0·127	0·109
„ 21	109½	3·15	8·93	0·75	0·128	0·108
„ 28	105	2·95	8·96	0·76	0·128	0·107
Apr. 4	102	3·10	8·87	0·78	0·130	0·109
„ 11	101	3·12	8·84	0·78	0·131	0·106
„ 18	103¾	2·92	8·92	—	—	—
„ 25	156	3·05	8·98	0·79	0·130	0·111
May 2	187¾	2·90	8·97	0·77	0·128	0·109
„ 9	194½	2·70	9·11	0·77	0·127	—
„ 16	189½	2·80	9·05	0·76	0·123	0·104
„ 23	185¼	2·65	8·89	0·75	0·119	0·100
„ 30	183¼	2·82	8·96	0·76	0·121	0·104
June 6	178	2·85	8·93	0·75	0·118	0·104
„ 13	174¼	3·05	8·90	0·76	0·121	0·102
„ 20	156¾	3·15	8·84	0·76	0·126	0·099
„ 27	150¾	3·18	8·82	0·76	0·125	0·100
July 4	137¾	3·22	8·52	0·76	0·120	0·094
„ 11	135¾	3·00	8·64	0·78	0·121	0·098
„ 18	127½	3·22	8·64	0·76	0·121	0·095
„ 25	126	3·18	8·60	0·77	0·121	0·097
Aug. 1	117½	3·20	8·57	0·76	0·122	0·095
„ 8	112½	3·50	8·32	0·77	0·118	0·093
„ 15	101	3·25	8·38	0·77	0·115	0·090
„ 22	129¼	3·68	8·46	0·76	0·123	0·093
„ 29	122	3·55	8·31	0·76	0·118	0·090
Sept. 5	119½	3·28	8·46	0·77	0·123	0·093
„ 12	114¾	3·52	8·52	0·79	0·124	0·089
„ 19	130¼	2·98	8·62	0·78	0·121	0·094
„ 26	125	2·85	8·60	0·79	0·122	0·094
Oct. 3	126	3·08	8·72	0·78	0·124	0·098
„ 10	174¾	3·18	8·72	0·79	0·127	0·098
„ 17	—	—	—	0·78	0·121	0·102

It is interesting to note that the highest value during 1932 was 0·131 per cent. on April 11, with 0·130 per cent. on April 4 and 25. This "peak" in the calcium curve in April has been observed during previous years, and the possibility that it was due to the change from winter to summer feeding was suggested. To investigate this a short intensive study of various properties of the milk was made during March to May 1932, but definite conclusions were not reached, since although the highest values were given when grass was

included in the ration yet there was an increase in early April while the herd was still on full winter rations. This point needs further investigation.

It seems highly probable that the low calcium values for 1932–3 particularly from May onwards are associated with the low total solids or solids-not-fat found during this period. The apparent relationship is obvious from Table V and Chart 2 G; while a study of the correlation coefficients between the calcium and the solids-not-fat during these two periods shows definite positive values. The general form of the curves shows such agreement that a further statistical investigation seems justified.

The possible association of these properties was suggested in Part I of this paper (1), p. 69).

Phosphorus.

During 1931–2 the highest and lowest values were 0·118 and 0·110 per cent. For 1932–3 these values were 0·112 and 0·089 respectively. From the figures in Table V or Chart 2 H it is obvious that the higher values in 1931–2 were all found during the months of November, December, January and February. The period 1932–3 is particularly interesting as extremely low values were found during the last four months of the experimental period, viz. June–September 1933. The similarity in type of the curve for phosphorus and that of the non-fatty solids (Chart 2 H and J) is very striking. From this, and from preliminary calculations it appears probable that a reduction in the phosphorus content is particularly associated with the very low non-fatty solids. A relationship is confirmed by a calculation of the correlation coefficients which show a positive correlation between these two factors.

During the first experimental period of 1928–9, low values for phosphorus from July to September were considered to be due to the drying off of certain cows in the herd, and associated with a supplemented grazing period; in 1929–30 an association with a supplemented grazing period but not with lowered milk yield was postulated; while in 1930–1 no wide variations were found but it was noted that ample pasture was available and no additional feeds were necessary until September. In 1931–2 a noticeable drop in the phosphorus was seen early in March although the cows were still receiving a winter ration, and after May 11 no supplementary feeding in addition to pasture was necessary until late September. From these considerations it is obvious that the apparently seasonal variations in phosphorus are probably due not to one but to several contributory causes which have not yet been disentangled. That the exceptionally low values in late summer of 1933 are intimately associated with the factors causing the low solids-not-fat seems however to be likely.

Summary.

This paper contains the results obtained in the fourth and fifth years of a study of the milk of a typical herd of healthy Shorthorn cows.

The period of drought in the summer of 1933 is included and furnishes

analyses which differ so much from the four previous years that the daily figures are given in Chart 1. In the earlier paper (1) weekly averages only could be published. The greatest seasonal variations which have been demonstrated by the daily analyses are:

(1) A fall of the percentage of fat in the morning's milk in the spring when the cows were on young grass. This was clearly shown in each of the five years.

(2) A fall in the percentage of solids-not-fat in the evening's and morning's milk during the period of drought in 1933, to an extent which had not previously been recorded.

The causes of these changes in the composition of milk will be further studied using the milk of individual cows.

The weekly analyses of morning's milk have also shown seasonal variations.

Relatively high values of the titratable acidity, formol titration, ash, calcium and phosphorus were observed from October 1931 to February 1932, followed by a general decrease in the summer months, while in the late autumn somewhat higher values were again found.

During the summer and early autumn of 1933 values which were lower than any previously obtained during this investigation were recorded for formol titration and for phosphorus, while the time of coagulation with rennet was longer.

Preliminary consideration of the data has revealed the possibility that the fall in non-fatty solids may be particularly associated with the phosphorus content.

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REFERENCES.

(1) GOLDING, MACKINTOSH and MATTICK (1932). *J. Dairy Res.* **4**, 48.
(2) GOLDING (1934). *Analyst*, **59**, 468.
(3) HORTVET (1921). *J. industr. Engng Chem.* **13**, 198.
(4) BIILMAN (1921). *Ann. Chim. (Phys.)*, **9**, XVI, 109.

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91. A STATISTICAL EXAMINATION OF THE INTER-RELATIONSHIP AND VARIABILITY OF PLATE COUNT, PRESUMPTIVE COLIFORM CONTENT AND KEEPING QUALITY OF RAW MILK

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(With 3 Charts.)

The keeping quality of milk may be defined as a measure of the period during which chemical changes due to the action of micro-organisms remain insufficient to be unpleasant to the sense of taste and/or smell. It is important to know to what extent the practice of hygienic methods, resulting in the exclusion of micro-organisms, affects the rate of deterioration of milk. It may also be asked whether our laboratory methods of growing and counting bacteria measure, in fact, those organisms which cause deterioration in milk. It is also desirable to know whether milk gains in keeping quality as bacteria are excluded to the point when numbers become minimal. A test for keeping quality aims at a measure of the gross effects of the bacterial population at a given temperature, and is therefore likely to prove of assistance in solving such problems. Hoy (1) devised a routine method of estimating the keeping quality of milk. The milk was tasted and simultaneously its heat stability at the boiling-point of water measured.

The writer has investigated several aspects of this test and work has also been done in America. Thus in 1927 Barkworth *et al.* (2) showed that coliform organisms, apart from the total bacterial content, had a deleterious effect on the keeping quality, and Hoy and Newland (3) have indicated that this is equally true of pasteurised milk. In America, Robertson and Frayer (4) made a statistical estimate of the relationship between bacterial content and keeping quality. They came to the conclusion that the plate count (as compared with the methylene blue reductase test, microscopic count and clump microscopic count) was the best interpreter of probable keeping quality in low-count milk. They also tested keeping quality at temperatures other than 60° F., and stressed the importance of using the logarithm of the plate count when dealing with relationships. Their work, however, makes no allowance for the presence of coliform organisms. Barkworth (5) has stated that the fat content has no appreciable effect on the keeping quality, but the findings of Thomas and Jones (6) do not agree with this. Barkworth (7) has also shown that the average keeping quality of samples of afternoon milk is 9 hours less than that of samples of morning milk of similar plate count. Samples of morning milk are

tested for plate count when 28 hours old, and samples of afternoon milk when only 24 hours old. The average keeping quality for samples of afternoon milk with a plate count, when 24 hours old, of 1000–2500 per ml. was found to be 71·8 hours. Samples of morning milk of similar plate count, made at 28 hours old, showed an average keeping quality of 81·0 hours, while an average keeping quality of 71·4 hours was found for samples of morning milk with a plate count, at 28 hours old, of 7500–10,000 per ml. If the keeping quality is accepted as the standard then samples of afternoon milk should show a plate count of the order of 10,000 per ml. when 28 hours old. Experimental work along these lines (8) did not suggest that the difference in the age on testing for plate count could account for the 9 hours' difference in average keeping quality, but in any case it is clear that results from samples of morning and afternoon milk must be tabulated separately whenever keeping quality is under consideration. An attempt (9) was made to estimate the variability of keeping quality and it was found that a given count (arithmetic) was associable with a keeping quality which would vary by 20 per cent. of the mean, a total range of variability of approximately 1 day (± 12 hours). This range is based on the coefficient of variability and would include $66\frac{2}{3}$ per cent. of the results. In this paper (9), while the individual plate counts were considered in terms of their arithmetic values the grouping system used approximated to logarithmic distribution. The grouping was, however, an unsuitable basis for full statistical treatment, and no special differentiation was made for the presence of coliform organisms.

Although several important points have been elucidated, there has as yet been no attempt to correlate the results of keeping-quality tests with those of the plate test for total count and the presumptive coliform test. Such a correlation would help to determine the relative importance of coliform organisms and total bacterial content in determining the keeping quality, and provide data on the variability of each factor at different levels. The data reported include those samples already examined (9), but many more have been added.

SOURCE OF SAMPLES.

The results tabulated were obtained almost exclusively from samples submitted during successive clean milk competitions in the counties of Kent, Surrey, East and West Sussex during the years 1923–32. The samples were taken from churns, at the end of milking, and sent to the laboratory by post. A few private samples from farms in the same area have been included. The period covers thirty-one competitions with a total of over 1500 competitors, and more than 12,000 samples of which over 10,000 fall below the maximum selected for this work, *i.e.* 500,000 per ml. These samples came from approximately 700 different farms. No attempt has been made to equalise the number of samples from each farm or from any one farm in any year, but the large number of different farms and the change of seasons should guarantee a wide variation in the nature of the flora of the samples. It should be noted that in

each year the great majority of samples were received in the period January–June, when excessive temperatures during transit are unusual. The temperature conditions during transit are, of course, unknown, but the arrival temperature at 8.30 a.m. was recorded in nearly all cases. In the earlier survey (9) the samples were divided into two groups according to their arrival temperatures, above and below 60° F., but it was not found that the higher arrival temperatures affected the relationship between plate count and keeping quality. At high temperatures bacteria multiply quicker, a sample has a higher plate count at a given age and a shorter keeping quality, but the keeping quality is not disproportionate to the plate count.

Technique.

The standard technique of the Ministry of Agriculture (10) was used throughout. Since 1923 certain improvements have been incorporated, but the media remained essentially the same. Until January 1929 the reaction of the agar was adjusted to +0·5 on Eyre's scale, but since then adjustments have been made colorimetrically to *p*H 6·8. Incubation for 48 hours at 37° C. has been used throughout when estimating the plate count; dilution has always been into 9 ml. saline blanks, and the medium for the presumptive coliform test has remained unchanged. The keeping-quality test has also remained unaltered, although in 1929 a change, which is discussed later, was made in the system of recording results. Samples of morning milk were about 28 hours old and samples of afternoon milk about 24 hours old when tested for plate count.

Classification of data.

Plate count.

Robertson and Frayer (4) have presented in some detail the reasons why logarithmic values should be used in comparing plate counts. On an arithmetic basis class intervals of statistically admissible equality are impossible. The logarithm (to four figures) of each count was therefore recorded. A class interval of log. 0·3000 was selected. With counts up to 500,000 per ml. this gives twelve classes, a figure previously shown to be very suitable.

For statistical purposes it is convenient to assume that the first class is log. 2·1–2·4 with mid-log. at 2·25. In effect this means that all the samples whose plate counts were under 125 per ml., *i.e.* log. 0·0–2·1, are assumed to be evenly distributed over the range 125–250 per ml. (log. 2·1–2·4). While admittedly an adjustment, it would appear not to introduce a material error. The samples so affected are shown in Table I.

From the table it will be seen that about half the samples at this low count are falsely classified, though for practical purposes it would be fallacious to distinguish plate counts below 125 per ml. (log. 2·1). A few samples, 0·146 per cent. of the gross total of 10,305 samples, gave no growth on the plates.

Table I. *First group (logarithm of plate count, 0–2·4); to show numbers of samples correctly and incorrectly treated: (a) actual range of group log. 0–2·4; (b) nominal range of group for statistical work log. 2·1–2·4.*

Class	Morning milk No. of samples Logarithm of plate count		Afternoon milk No. of samples Logarithm of plate count	
	2·1 or less	2·1–2·4	2·1 or less	2·1–2·4
Coliform organisms:				
Absent from 1 ml.	217*	157	160†	165
Present in 1 ml.	9	11	12	7
,, 1/10 ml.	1	8	2	3
,, 1/100 ml.	—	1	1	—
,, 1/1000 ml.	—	—	—	—
Total	227	177	175	175
Total of group	404		350	

* Includes 6 samples showing no growth on plates.

† Includes 9 samples showing no growth on plates.

Presumptive coliform test.

In this test quantities of 1, 1/10, 1/100 and 1/1000 ml. of milk are added to lactose-litmus-bile-salt-peptone broth and the production of acid and gas is regarded as a positive reaction. It is well known that *Bact. coli* is not the only organism that will grow under these conditions, and it is therefore preferable to employ the term "coliform organisms." Data showing only "presence" or "absence" are not so amenable to class grouping and statistical treatment as the plate counts, which are definite enumerations, but it may be noted that the intervals are logarithmic. The results are based on single tests at each dilution, and on probability it may be assumed that the true intensity of contamination will be sometimes greater and sometimes less than the theoretical intensity, and it was decided to regard the decimal fractions (1, 1/10, 1/100, 1/1000 ml.) as the mid-class points of five logarithmic stages of coliform contamination, with a class interval of unity. Thus the assumption is made that the first group "absent from 1 ml." is a definite group defined as "present in 10 ml." The effect of this assumption will be discussed later.

Keeping quality.

The unit of classification finally selected is the $\frac{1}{2}$ day, but grouping of results of keeping-quality tests is far more complex than the simple statement would suggest. The difficulties connected with the interpretation of keeping-quality estimations were indicated by Barkworth in 1929 (11). The figures here presented cannot be appreciated at their correct value without some knowledge of the steps by which keeping-quality results are eventually grouped into $\frac{1}{2}$ days. For classification purposes we have the choice of three units: the $\frac{1}{2}$ day, because the samples are tasted twice daily; the $\frac{1}{4}$ day or the hour, because these are the two units used in recording the results of keeping-quality tests.

The following explanation, together with Table II, will serve to elucidate the relationship of these units to one another.

For routine purposes, the tasting times have been standardised at 9.0 a.m. and 5.0 p.m. (Table II, line 1), the sample being recorded as "sweet" on each occasion, provided it is heat-stable and that the flavour is unimpaired. Either clot on immersion in a tube in boiling water or definite unpalatability ends the life of the sample. A full score is given for each "sweet" period, but nothing for the occasion of failure. There will, however, be the case when the sample though heat-stable is mildly off-flavoured but not unpalatable, and here the practice is to record a "taint" and to regard the period as only half that of a fully "sweet" period. If the twice daily tasting times are considered as dividing the day of 24 hours into two periods called $\frac{1}{2}$ days, and if we assume that the "taint" ends the life exactly half-way between the two tasting times, then it follows that the recording of a "taint" introduces a $\frac{1}{4}$-day period. As the tasting times are not at 12-hour intervals, the $\frac{1}{4}$ days cannot be regarded as equal 6-hour periods and are usually referred to as *nominal $\frac{1}{4}$ days* (Table II, lines 2, 5, 7 and 10). It will be seen that whereas the end of a $\frac{1}{4}$-day period falls at the middle of the short lap of 8 hours in the case of morning milk, it falls at the middle of the long interval of 16 hours in the case of afternoon milk. This relative position of morning and afternoon milks is reversed at the end of a $\frac{3}{4}$-day period when the middle of the longer interval falls to the lot of morning milk. That this cross-over does not have a counterbalancing effect will be realised when we consider the interval in hours between the milking time and the end of the life. This period is called the *true life*, and comparing true life and nominal $\frac{1}{4}$ days we have the following:

	Nominal $\frac{1}{4}$ days	
True life	Afternoon milks	Morning milks
18 hours	$\frac{1}{2}$ day	$\frac{3}{4}$ day
42 „	$1\frac{1}{2}$ days	$1\frac{3}{4}$ days

A similar situation will obviously occur at corresponding periods throughout the life of the milks. The reason for this state of affairs is that the fixed tasting times, 9.0 a.m. and 5.0 p.m., stand in unequal relationship to the milking times, 6.30 a.m. and 3.30 p.m., normal to the area.

In the keeping-quality test we therefore start by tasting twice daily, endeavour to obtain a closer measure by introducing the factor of the "taint," and finally introduce the "true life" which, by measuring in hours, aims at eliminating the inequalities of the nominal $\frac{1}{4}$ days and the relationship to the milking times. It might be thought that the use of the true life system would enable us to use groups of equal 6-hour periods, provided that the grouping was such that all samples failing in the keeping-quality test at a given tasting time were included in the same hour group. If a morning sample with a life of $\frac{1}{4}$ day in "nominal $\frac{1}{4}$ days" is recorded as 7 hours in "true life," any system of grouping in hours by equal intervals should be such that the first period

Table II. *Relationship of tasting times, nominal age and true life, and comparison of morning and afternoon milks.*

	Line		Tasting time					
	1		9.0 a.m.	5.0 p.m.	9.0 a.m.	5.0 p.m.	9.0 a.m.	
			4 hours + *4* hours	*8* hours + *8* hours	*4* hours + *4* hours	*8* hours + *8* hours	*4* hours	
			8 hours interval	16 hours interval	8 hours interval	16 hours interval		
Afternoon milk Milking time 3.0 p.m.	2	Nominal age	½ day (12 hours)	1 day (24 hours)	1½ days (36 hours)	2 days (48 hours)		
	3							
	4	True life (interval since milking)	**18** hours	*26* hours	**42** hours	*50* hours		
	5		¾ day	1¼ days	1¾ days			
	6		26 hours less 4 =*22* hours	42 hours less 8 =*34* hours	50 hours less 4 =*46* hours			
Morning milk Milking time 7.0 a.m.	7	Nominal age		½ day (12 hours)	1 day (24 hours)	1½ days 36 hours	2 days (48 hours)	
	8							
	9	True life (interval since milking)		*10* hours	*26* hours	*34* hours	*50* hours	
	10			¾ day	1¼ days	1¾ days		
	11			26 hours less 8 =**18** hours	34 hours less 4 =*30* hours	50 hours less 8 =**42** hours		

includes the 7-hour point. Six-hour periods which satisfy these conditions can only be achieved as follows:

Table II A

	Morning milk (*a*)	Afternoon milk (*b*)
¼ day	3– 8½ hours	7½–12 hours
½ „	9–14½ „	12½–19 „
¾ „	15–20½ „	19½–24 „
1 „	21–26½ „	24½–31 „

An objection to this is that true life is determined from the milking time, and a survey of milking times in the area from which the samples are drawn (12) shows that, reckoning by ½-hour periods, 60 per cent. of the herds are milked at 6.0 a.m. and the remainder over the period 4.30–8.0 a.m. For afternoon milkings the spread is from 1.30 to 7.30 p.m., and 76 per cent. fall at 3.0 or 3.30 or 4.30 p.m. Taking as an example the morning milking time of 6.0 a.m., the true life period for ¼ day is 7 hours, and therefore in the ¼-day group, 3–8½ hours, for morning milks, 60 per cent. of the samples would record 7 hours. In other words although true life does enable us to devise equal 6-hour groups, the individual records do not show normal distribution. For individual samples true life is an advantage because it does give the sample credit for the extra life attained at uneven tasting times, and it also, in part, evens up the apparent difference between the average keeping quality for samples of morning and of afternoon milk of similar plate count (7). Basically, true life is, however, merely a method of interpreting the results of keeping-quality tests, and as the "true life" of a sample is itself still based on the time value given to a "taint," from a statistical point of view the irregularities of interval of the nominal ¼ days are only reproduced in another form, with a slight added spread due to variations in milking time.

One last point arises from the connection between true life and milking time. In the group Table II A (*a*) and (*b*) above it will be noticed that the first group starts at 3 hours for morning milk and 7½ hours for afternoon milk, a rather large difference considering that the same stage of life of the sample is represented. If we abandon the ¼ day and use a 12-hour group it is possible to group the morning samples as in Table II B below. This brings the group figures nearer to those for the corresponding stage of afternoon milk but cannot be divided into ¼-day groups because the dividing figure, 10½ hours (5–10½ and 11–14½), is unsuitable. The true life period for ½ day for a sample milked at 6.30 a.m. is 10½ hours, and thus samples timed from this or later milking times would be placed in the ¼-day instead of the ½-day group. By ignoring the ¼-day division we circumvent this objection, and because the group figures are nearer to the corresponding group for afternoon samples this grouping (Table II B) has been used when calculating the deviation in *hours* (see Tables VI and VII).

Table II B

Morning samples	Afternoon samples
½ day 5–16½ hours	7½–19 hours
1 „ 17–28½ „	19½–24 „

Summing up we see that the results of keeping-quality tests are recorded either as $\frac{1}{4}$ days (nominal $\frac{1}{4}$ days) or as hours (true life), being founded on a twice-daily testing with an optional estimation of failure to obtain full score at the $\frac{1}{2}$-day test (taint). To call the taint a $\frac{1}{4}$ day is a convention, and the true position and even appraisement of the taint is a matter of opinion. It has been shown that the $\frac{1}{4}$ days are not truly equal periods, and that the system of recording by hours merely reproduces these irregularities in another form, so that, finally, we are left with the original twice-daily testing for a classification basis. This gives us half a day as the unit, but no particular period of hours is attached to the $\frac{1}{2}$ day, nor is any fixed point regarded as the mid-point of any $\frac{1}{2}$-day period. Such is impossible owing to the irregularities of the testing times and their relationship to the various morning and afternoon milking times. In employing this grouping those milks which are recorded as "tainted" at any particular time are grouped with those that pass as fully sweet at the same tasting. We have to choose whether a mild off-flavour is to class as sweet or as condemned, and inasmuch as a taint is considered still a palatable stage it seems justified to group with the fully sweet milks. It will be realised that whatever basis is employed, keeping-quality records are untractable data, and as there are indications(9) that the variability is in the order of a half a day it is considered that the basis finally selected is the most suitable.

TABULATION.

As there are three variates there are three relationships to be examined; moreover, it has been indicated above that it is necessary to consider morning and afternoon milks in separate categories. The arrangement is therefore as follows, each individual tabulation being prepared for morning and afternoon milks. In each of the three main tables as outlined below x refers to horizontal and y to vertical values both in tables and graphs.

(1) *Plate count and keeping quality.* x = log. plate count, 12 stages. y = keeping quality, 13 stages. Besides the main table it is possible to prepare five subtables for each level of coliform contamination.

(2) *Coliform content and keeping quality.* x = coliform content, 5 stages. y = keeping quality, 13 stages. The main table is divisible into twelve subtables, one for each level of log. plate count.

(3) *Coliform content and plate count.* x = coliform content, 5 stages. y = log. plate count, 12 stages. There is a main table and thirteen subtables for the individual $\frac{1}{2}$-day periods.

RESULTS.

The results fall under two heads, relationship and variability, which will be dealt with separately. For plate count and keeping quality the six tables have been summarised in Tables III and IV, while the results from the three main tables are shown graphically in Charts 1, 2 and 3, and the means are shown in Table V. Variability of x for given y and y for given x is seen in

Table III. *Plate count, presumptive coliform content and keeping quality* (60° F.) (*morning milk*).

Group No.	Plate count: Logarithm	Plate count: Approx. arithmetic count (up to)		Average keeping quality (half-days): Coliform organisms: Absent from 1 ml. — log. 10 = 1·00	Present in 1 ml. — log. 1 = 0·00	Present in 1/10 ml. — log. 0·1 = − 1·00	Present in 1/100 ml. — log. 0·01 = − 2·00	Present in 1/1000 ml. — log. 0·001 = − 3·00	All samples combined
				Correlation and variation value					
1	0·0000–2·4000	250	(*a*)	374	20	9	1	—	404
	(mid-log. 2·25)		(*b*)	**7·57**	**6·80**	**6·44**	**7·00**	—	**7·50**
			(*c*)	*88·3*	*79·2*	*73·4*	*82·5*	—	*87·5*
2	2·4001–2·7000	500	(*a*)	205	24	10	3	—	242
	(mid-log. 2·55)		(*b*)	**7·24**	**6·71**	**6·10**	**6·00**	—	**7·13**
			(*c*)	*84·7*	*78·2*	*70·3*	*67·8*	—	*83·3*
3	2·7001–3·0000	1,000	(*a*)	282	41	14	4	—	341
	(mid-log. 2·85)		(*b*)	**7·16**	**6·56**	**6·21**	**6·50**	—	**7·04**
			(*c*)	*83·8*	*76·9*	*73·2*	*72·4*	—	*82·4*
4	3·0001–3·3000	2,000	(*a*)	337	65	34	12	4	452
	(mid-log. 3·15)		(*b*)	**6·97**	**6·40**	**6·21**	**5·92**	**6·00**	**6·80**
			(*c*)	*81·6*	*74·3*	*71·6*	*67·7*	*68·6*	*79·4*
5	3·3001–3·6000	4,000	(*a*)	307	94	49	20	7	477
	(mid-log. 3·45)		(*b*)	**6·86**	**6·32**	**6·16**	**5·85**	**5·14**	**6·61**
			(*c*)	*80·4*	*73·6*	*71·0*	*67·9*	*60·4*	*77·3*
6	3·6001–3·9000	8,000	(*a*)	376	123	81	44	15	639
	(mid-log. 3·75)		(*b*)	**6·54**	**6·18**	**6·06**	**5·55**	**5·26**	**6·29**
			(*c*)	*76·3*	*72·5*	*68·7*	*65·1*	*60·6*	*73·5*
7	3·9001–4·2000	16,000	(*a*)	258	132	116	79	30	615
	(mid-log. 4·05)		(*b*)	**6·40**	**6·04**	**5·82**	**5·41**	**5·22**	**6·02**
			(*c*)	*74·8*	*70·5*	*67·8*	*62·6*	*60·2*	*70·3*
8	4·2001–4·5000	32,000	(*a*)	165	99	96	76	41	477
	(mid-log. 4·35)		(*b*)	**6·32**	**5·75**	**5·68**	**5·50**	**5·14**	**5·84**
			(*c*)	*73·7*	*66·2*	*66·3*	*63·6*	*59·8*	*67·8*
9	4·5001–4·8000	63,000	(*a*)	122	76	105	89	74	466
	(mid-log. 4·65)		(*b*)	**6·13**	**5·51**	**5·32**	**5·28**	**4·97**	**5·49**
			(*c*)	*71·5*	*64·3*	*61·8*	*59·9*	*57·1*	*63·6*
10	4·8001–5·1000	126,000	(*a*)	68	44	54	65	84	315
	(mid-log. 4·95)		(*b*)	**5·85**	**5·39**	**5·37**	**5·06**	**4·84**	**5·29**
			(*c*)	*69·2*	*62·3*	*62·5*	*58·4*	*56·0*	*61·4*
11	5·1001–5·4000	251,000	(*a*)	45	19	61	53	88	266
	(mid-log. 5·25)		(*b*)	**5·56**	**5·16**	**5·02**	**4·94**	**4·62**	**4·97**
			(*c*)	*64·6*	*60·6*	*58·3*	*57·9*	*53·3*	*57·8*
12	5·4001–5·7000	500,000	(*a*)	38	16	29	43	104	230
	(mid-log. 5·55)		(*b*)	**4·89**	**5·06**	**5·14**	**4·56**	**4·44**	**4·67**
			(*c*)	*56·5*	*57·9*	*60·1*	*53·3*	*50·7*	*53·8*
Total samples				2577	753	658	489	447	4924
Mean: Half-days				**6·46**	**5·99**	**5·79**	**5·63**	**5·07**	**6·14**
Hours				*75·4*	*69·7*	*67·1*	*64·9*	*58·5*	*71·5*
Unweighted mean, hours				*79·5*	*70·2*	*65·7*	*61·1*	*55·4*	*72·2*

(*a*) No. of samples; (*b*) mean in half-days; (*c*) mean in hours.

Table IV. *Plate count, presumptive coliform content and keeping quality* (60° F.) (*afternoon milk*).

Group No.	Plate count: Logarithm	Plate count: Approx. arithmetic count (up to)		Average keeping quality (half-days): Coliform organisms: Absent from 1 ml. — Correlation and variation value: log. 10 = 1·00	Present in: 1 ml. — log. 1 = 0·00	1/10 ml. — log. 0·1 = −1·00	1/100 ml. — log. 0·01 = −2·00	1/1000 ml. — log. 0·001 = −3·00	All samples combined
1	0·0000–2·4000 (mid-log. 2·25)	250	(*a*)	325	19	5	1	—	350
			(*b*)	**6·58**	**5·53**	**6·00**	**4·00**	—	**6·50**
			(*c*)	*79·6*	*67·4*	*69·5*	*46·0*	—	*78·7*
2	2·4001–2·7000 (mid-log. 2·55)	500	(*a*)	261	24	13	4	2	304
			(*b*)	**6·40**	**5·58**	**5·62**	**5·00**	**4·00**	**6·27**
			(*c*)	*77·0*	*68·4*	*66·8*	*63·5*	*49·8*	*75·5*
3	2·7001–3·0000 (mid-log. 2·85)	1,000	(*a*)	375	46	25	13	2	461
			(*b*)	**6·22**	**5·61**	**5·40**	**4·92**	**4·00**	**6·07**
			(*c*)	*74·9*	*67·6*	*64·7*	*59·8*	*47·3*	*73·0*
4	3·0001–3·3000 (mid-log. 3·15)	2,000	(*a*)	433	53	38	21	4	549
			(*b*)	**5·98**	**5·25**	**5·26**	**5·19**	**4·75**	**5·85**
			(*c*)	*72·6*	*63·2*	*64·5*	*61·5*	*57·0*	*70·6*
5	3·3001–3·6000 (mid-log. 3·45)	4,000	(*a*)	361	77	46	25	8	517
			(*b*)	**5·94**	**5·25**	**5·09**	**4·72**	**4·38**	**5·68**
			(*c*)	*71·4*	*63·8*	*61·6*	*56·7*	*53·8*	*68·4*
6	3·6001–3·9000 (mid-log. 3·75)	8,000	(*a*)	426	101	105	44	32	708
			(*b*)	**5·64**	**5·17**	**4·82**	**4·68**	**4·28**	**5·33**
			(*c*)	*68·0*	*62·0*	*58·4*	*56·6*	*51·8*	*64·3*
7	3·9001–4·2000 (mid-log. 4·05)	16,000	(*a*)	315	104	80	79	55	632
			(*b*)	**5·58**	**4·98**	**4·90**	**4·53**	**4·31**	**5·16**
			(*c*)	*67·3*	*60·2*	*58·8*	*54·8*	*51·6*	*62·1*
8	4·2001–4·5000 (mid-log. 4·35)	32,000	(*a*)	161	62	90	56	48	417
			(*b*)	**5·40**	**4·92**	**4·71**	**4·30**	**4·21**	**4·90**
			(*c*)	*65·5*	*60·0*	*56·8*	*52·6*	*50·9*	*59·4*
9	4·5001–4·8000 (mid-log. 4·65)	63,000	(*a*)	150	61	73	77	81	442
			(*b*)	**5·19**	**4·61**	**4·52**	**4·22**	**4·20**	**4·65**
			(*c*)	*62·8*	*55·5*	*54·5*	*51·0*	*50·6*	*56·1*
10	4·8001–5·1000 (mid-log. 4·95)	126,000	(*a*)	118	45	58	85	111	417
			(*b*)	**4·96**	**4·64**	**4·43**	**4·14**	**3·83**	**4·38**
			(*c*)	*60·0*	*56·2*	*53·8*	*49·8*	*45·9*	*52·9*
11	5·1001–5·4000 (mid-log. 5·25)	251,000	(*a*)	70	27	46	73	114	330
			(*b*)	**4·79**	**4·70**	**4·43**	**4·12**	**3·72**	**4·22**
			(*c*)	*58·2*	*56·2*	*53·8*	*50·1*	*45·2*	*51·1*
12	5·4001–5·7000 (mid-log. 5·55)	500,000	(*a*)	42	13	38	52	108	253
			(*b*)	**4·50**	**4·46**	**4·28**	**3·94**	**3·59**	**3·95**
			(*c*)	*53·4*	*53·1*	*50·5*	*47·8*	*43·5*	*47·6*
Total samples				3037	632	617	530	565	5381
Mean: Half-days				**5·60**	**5·06**	**4·97**	**4·48**	**4·12**	**5·25**
Hours				*67·7*	*61·1*	*59·5*	*56·1*	*49·8*	*63·3*
Weighted mean, hours				*70·7*	*61·2*	*57·6*	*52·6*	*47·5*	*63·9*

(*a*) No. of samples; (*b*) mean in half-days; (*c*) mean in hours.

Table V. *Table of means.*

(*a*) Plate count and keeping quality

Logarithm of count	Mean keeping quality (half-days)	
	Morning milk	Afternoon milk
2·25	7·50	6·50
2·55	7·13	6·27
2·85	7·04	6·07
3·15	6·80	5·85
3·45	6·61	5·68
3·75	6·29	5·33
4·05	6·02	5·16
4·35	5·84	4·90
4·65	5·49	4·65
4·95	5·29	4·38
5·25	4·97	4·22
5·55	4·67	3·95

(*b*) Coliform organisms and keeping quality

Coliform organisms	Mean keeping quality (half-days)	
	Morning milk	Afternoon milk
Absent from 1 ml.	6·80	5·86
Present in 1 ml.	6·03	5·06
„ 1/10 ml.	5·66	4·77
„ 1/100 ml.	5·27	4·35
„ 1/1000 ml	4·81	3·93

(*c*) Coliform organisms and plate count

Coliform organisms	Mean logarithmic count	
	Morning milk	Afternoon milk
Absent from 1 ml.	3·42	3·47
Present in 1 ml.	3·90	3·89
„ 1/10 ml.	4·24	4·19
„ 1/100 ml.	4·49	4·49
„ 1/1000 ml.	4·90	4·83

Table VIII. *Summary of variabilities.*

Type	Morning milk		Afternoon milk	
	Standard deviation	Coefficient of variability %	Standard deviation	Coefficient of variability %
Keeping quality:				
On logarithmic plate count	1·137 half-days	18·5	1·130 half-days	21·5
On logarithmic coliform content	1·220 „	21·4	1·130 „	23·6
Logarithmic plate count:				
On keeping quality	0·75 log.	21·6	0·77 log.	21·1
On logarithmic coliform content	0·76 „	17·9	0·77 „	18·3
Logarithmic coliform content:				
On keeping quality	1·167 log.	1060·9	1·174 log.	117·4
On logarithmic plate count	1·123 „	623·9	1·174 „	903·1

Section	Count and k
1a	Keeping quality on co (half-days)
1b	Count on keeping qual (logarithms)
	Coliform and
2a	Keeping quality on co (half-days)
2b	Coliform on keeping q (logarithms)
	Colifo
3a	Count on coliform (logarithms)
3b	Coliform on count (logarithms)

Table VI. *Variability*

		Coliform organisms			
		Absent from 1 ml. (1) log. 1·00	Present in 1 ml. (2) log. 0·00	Present in 1/10 ml. (3) log. −1·00	Pre
	No. of samples ...	2577	753	658	
Count and keeping quality				Mean of 12 levels of logarithm count	
quality on count	σ	1·198	0·996	0·947	
ays)	Mean	**6·46**	**5·99**	**5·79**	
	v%	*18·5*	*16·6*	*16·4*	
	v% (hours)	(*19·1*)	(*17·1*)	(*16·9*)	
	Mean (hours)	**(75·4)**	**(69·1)**	**(67·1)**	
				Mean of 13 levels of half-day keeping quality	
keeping quality	σ	0·74	0·67	0·68	
hms)	Mean	**3·69**	**3·99**	**3·96**	
	v%	*20·1*	*16·9*	*17·1*	

		Logarithm count groups							
	Mid-logarithm ...	2·25	2·55	2·85	3·15	3·45	3·75	4·05	4·35
		1	2	3	4	5	6	7	8
	No. of samples ...	404	242	341	452	477	639	615	477
Coliform and keeping quality						Mean of 5 levels of coliform content			
quality on coliform	σ	1·265	1·170	1·177	1·166	1·143	1·045	1·072	1·052
ays)	Mean	**6·95**	**6·51**	**6·61**	**6·30**	**6·08**	**5·89**	**5·78**	**5·68**
	v%	*18·2*	*18·0*	*17·8*	*18·5*	*18·8*	*17·7*	*18·5*	*18·5*
						Mean of 13 levels of half-day keeping quality			
on keeping quality	σ	0·387	0·560	0·571	0·791	0·895	1·078	1·172	1·268
hms)	Mean	**0·92**	**0·70**	**0·85**	**0·73**	**0·27**	**0·48**	**0·15**	**−0·19**
	v%	*42·0*	*80·0*	*67·2*	*103·4*	*331·5*	*224·6*	*781·3*	*667·4*
	Mean (ml.)	**8·32**	**5·01**	**7·08**	**5·37**	**1·86**	**3·02**	**1·41**	**0·65**

		Half-days								
		1	2	3	4	5	6	7	8	9
	No. of samples ...	—	2	68	320	1265	1291	1256	472	19
Coliform on count						Mean of 5 levels of coliform content				
coliform	σ	—	—	0·48	0·63	0·69	0·72	0·70	0·59	0·
hms)	Mean	—	**5·10**	**5·03**	**4·62**	**4·40**	**4·11**	**3·84**	**3·80**	**3·**
	v%	—	—	*9·5*	*13·6*	*15·7*	*17·6*	*18·3*	*15·6*	*19·*
						Mean of 12 levels of logarithm count				
on count	σ	—	—	1·619	1·360	1·303	1·129	0·852	0·502	0·
hms)	Mean	—	**−1·00**	**−1·55**	**−0·61**	**−0·47**	**−0·07**	**0·31**	**0·63**	**0·**
	v%	—	—	*104·5*	*223·0*	*277·2*	*1612·9*	*274·8*	*79·7*	*36·*
	Mean (ml.)	—	**0·1**	**0·03**	**0·25**	**0·34**	**0·85**	**2·04**	**4·27**	**5·**

Table VI. *Variability (morning milk).*

	Coliform organisms			
sent in 1 ml.	Present in 1/10 ml.	Present in 1/100 ml.	Present in 1/1000 ml.	Main
) log. 0·00	(3) log. −1·00	(4) log. −2·00	(5) log. −3·00	tables
753	658	489	447	4924
	Mean of 12 levels of logarithm count			
0·996	0·947	0·888	0·852	1·137
5·99	**5·79**	**5·63**	**5·07**	**6·14**
16·6	*16·4*	*15·8*	*16·8*	*18·5*
(17·1)	*(16·9)*	*(16·4)*	*(17·5)*	*(19·1)*
(69·1)	**(67·1)**	**(64·9)**	**(58·5)**	**(71·5)**
	Mean of 13 levels of half-day keeping quality			
0·67	0·68	0·61	0·53	0·75
3·99	**3·96**	**4·53**	**4·71**	**3·48**
16·9	*17·1*	*13·4*	*11·3*	*21·6*

Logarithm count groups									
3·15	3·45	3·75	4·05	4·35	4·65	4·95	5·25	5·55	
4	5	6	7	8	9	10	11	12	
452	477	639	615	477	466	315	266	230	4924
			Mean of 5 levels of coliform content						
1·166	1·143	1·045	1·072	1·052	0·908	1·038	1·007	0·945	1·22
6·30	**6·08**	**5·89**	**5·78**	**5·68**	**5·43**	**5·32**	**5·06**	**5·06**	**5·71**
18·5	*18·8*	*17·7*	*18·5*	*18·5*	*16·7*	*19·5*	*19·9*	*18·7*	*21·4*
			Mean of 13 levels of half-day keeping quality						
0·791	0·895	1·078	1·172	1·268	1·305	1·397	1·370	1·454	1·167
0·73	**0·27**	**0·48**	**0·15**	**−0·19**	**−0·75**	**−0·60**	**−0·59**	**−1·05**	**0·11**
108·4	*331·5*	*224·6*	*781·3*	*667·4*	*174·0*	*232·8*	*232·0*	*138·5*	*1060·9*
5·37	**1·86**	**3·02**	**1·41**	**0·65**	**0·18**	**0·251**	**0·257**	**0·09**	**1·29**

	Half-days								
	5	6	7	8	9	10	11	12	13	
	1265	1291	1256	472	193	43	18	3	3	4924
				Mean of 5 levels of coliform content						
63	0·69	0·72	0·70	0·59	0·63	0·64	0·65	0·96	0·63	0·76
32	**4·40**	**4·11**	**3·84**	**3·80**	**3·32**	**2·60**	**2·72**	**4·15**	**2·65**	**4·24**
6	*15·7*	*17·6*	*18·3*	*15·6*	*19·0*	*24·7*	*24·1*	*23·1*	*23·7*	*17·9*
				Mean of 12 levels of logarithm count						
360	1·303	1·129	0·852	0·502	0·271	0·102	—	—	—	1·122
81	**−0·47**	**−0·07**	**0·31**	**0·63**	**0·75**	**0·98**	**1·0**	**1·0**	**1·0**	**−0·18**
0	*277·2*	*1612·9*	*274·8*	*79·7*	*36·1*	*10·4*	—	—	—	*623·9*
25	**0·34**	**0·85**	**2·04**	**4·27**	**5·62**	**9·55**	**10·0**	**10·0**	**10·0**	**0·83**

Main tables 4924	Remarks
1·137 **6·14** *18·5* *(19·1)* **(71·5)**	σ decreases slightly and v increases as logarithm increases (cf. Section 2*a*)
0·75 **3·48** *21·6*	Few samples at extremes of keeping quality; v increases with keeping quality, up 26% at 13 half-days (cf. Section 3*a*) 3000 per ml. might be 540–17,000 per ml. (to include 66⅔% of result)
4924	
1·22 **5·71** *21·4*	Both σ and v improve as coliform increases (cf. Section 1*a*)
1·167 **0·11** *1060·9* **1·29**	Most samples 5–7 half-days. v is lower at either extreme (cf. Section 3*b*) +1 ml. might be "abs." or +1/10 ml. (to include 66⅔% of result)
4924	
0·76 **4·24** *17·9*	See remarks, Section 2*a*
1·122 **−0·18** *623·9* **0·83**	σ increases 0·4 to 1·5 as logarithm increases (cf. Section 2*b*)

Section	Count and keeping q
1a	Keeping quality on count (half-days)
1b	Count on keeping quality (logarithms)
	M
	N
	Coliform and keeping
2a	Keeping quality on coliform (half-days)
2b	Coliform on keeping quality (logarithms)
	N
	Coliform and co
3a	Count on coliform (logarithms)
3b	Coliform on count (logarithms)

Table VII. *Variability (afterno*

		Coliform organisms			
		Absent from 1 ml. (1) log. 1·00	Present in 1 ml. (2) log. 0·00	Present in 1/10 ml. (3) log. −1·00	Present in 1/ (4) log. −
	No. of samples ...	3037	632	617	530
nd keeping quality		Mean of 12 levels of logarithm count			
on count	σ	1·164	0·949	0·940	0·82
	Mean	**5·6**	**5·06**	**4·97**	**4·48**
	v%	*20·8*	*18·8*	*18·9*	*18·4*
	v% (hours)	*(20·6)*	*(18·6)*	*(19·0)*	*(18·3)*
	Mean (hours)	**(67·7)**	**(61·1)**	**(59·5)**	**(54·2)**
		Mean of 13 levels of half-day keeping quality			
g quality	σ	0·75	0·75	0·72	0·69
	Mean	**3·38**	**4·07**	**4·19**	**4·47**
	v%	*22·3*	*18·5*	*17·2*	*15·4*

		Logarithm count groups								
	Mid-logarithm ...	2·25	2·55	2·85	3·15	3·45	3·75	4·05	4·35	4·65
		1	2	3	4	5	6	7	8	9
	No. of samples ...	350	304	461	549	517	708	633	417	442
and keeping quality		Mean of 5 levels of coliform content								
on coliform	σ	1·283	1·148	1·181	1·064	1·063	0·998	0·940	1·057	0·9
	Mean	**5·45**	**5·30**	**5·23**	**5·30**	**5·07**	**4·92**	**4·90**	**4·70**	**4·5**
	v%	*23·5*	*21·7*	*22·6*	*20·1*	*21·0*	*21·3*	*19·2*	*22·5*	*20·9*
		Mean of 13 levels of half-day keeping quality								
ing quality	σ	0·357	0·629	0·707	0·803	0·878	1·082	1·148	1·274	1·4
	Mean	**0·87**	**0·71**	**0·63**	**0·56**	**0·54**	**0·17**	**−0·05**	**0·00**	**−0·2**
	v%	*41·0*	*88·6*	*112·2*	*143·4*	*162·6*	*636·5*	*2296·0*	*127·4*	*667·2*
	Mean (ml.)	**7·41**	**5·13**	**4·23**	**3·63**	**3·47**	**1·48**	**0·89**	**1·00**	**0·6**

		Half-days								
		1	2	3	4	5	6	7	8	9
	No. of samples ...	5	54	268	1290	1469	1382	620	234	40
liform and count		Mean of 5 levels of coliform content								
n	σ	0·42	0·56	0·59	0·65	0·76	0·74	0·72	0·71	0·53
	Mean	**5·03**	**4·81**	**4·71**	**4·40**	**4·08**	**3·90**	**3·43**	**3·66**	**2·82**
	v%	*8·4*	*13·8*	*12·6*	*14·7*	*18·5*	*19·0*	*21·0*	*19·3*	*18·6*
		Mean of 12 levels of logarithm count								
t	σ	1·414	1·283	1·435	1·422	1·200	0·816	0·458	0·227	—
	Mean	**−1·5**	**−0·84**	**−1·21**	**−0·58**	**−0·15**	**0·46**	**0·86**	**0·78**	**1·0**
	v%	*94·3*	*152·7*	*118·6*	*245·2*	*800·0*	*177·4*	*53·3*	*29·1*	—
	Mean (ml.)	**0·03**	**0·14**	**0·06**	**0·38**	**0·71**	**2·88**	**7·24**	**6·03**	**10·0**

Table VII. *Variability (afternoon milk).*

Coliform organisms

Present in 1 ml. (2) log. 0·00	Present in 1/10 ml. (3) log. −1·00	Present in 1/100 ml. (4) log. −2·00	Present in 1/1000 ml. (5) log. −3·00	Main tables
632	617	530	565	5381
Mean of 12 levels of logarithm count				
0·949	0·940	0·826	0·817	1·130
5·06	**4·97**	**4·48**	**4·17**	**5·25**
18·8	*18·9*	*18·4*	*19·8*	*21·5*
(18·6)	*(19·0)*	*(18·3)*	*(19·7)*	*(21·4)*
(61·1)	**(59·5)**	**(54·2)**	**(49·8)**	**(63·3)**
Mean of 13 levels of half-day keeping quality				
0·75	0·72	0·69	0·58	0·77
4·07	**4·19**	**4·47**	**4·89**	**3·65**
18·5	*17·2*	*15·4*	*11·8*	*21·1*

Logarithm count groups

3·15	3·45	3·75	4·05	4·35	4·65	4·95	5·25	5·55	
4	5	6	7	8	9	10	11	12	
549	517	708	633	417	442	417	330	253	5381
Mean of 5 levels of coliform content									
1·064	1·063	0·998	0·940	1·057	0·951	1·025	1·001	0·914	1·131
5·30	**5·07**	**4·92**	**4·90**	**4·70**	**4·55**	**4·47**	**4·33**	**4·13**	**4·79**
20·1	*21·0*	*21·3*	*19·2*	*22·5*	*20·9*	*22·9*	*23·1*	*22·1*	*23·6*
Mean of 13 levels of half-day keeping quality									
0·803	0·878	1·082	1·148	1·274	1·401	1·453	1·420	1·225	1·174
0·56	**0·54**	**0·17**	**−0·05**	**0·00**	**−0·21**	**−1·00**	**−0·95**	**−1·15**	**0·00**
143·4	*162·6*	*636·5*	*2296·0*	*127·4*	*667·2*	*145·3*	*149·5*	*106·5*	*117·4*
3·63	**3·47**	**1·48**	**0·89**	**1·00**	**0·62**	**0·1**	**0·11**	**0·07**	**1·00**

Half-days

4	5	6	7	8	9	10	11	12	13	
1290	1469	1382	620	234	40	16	2	—	1	5381
Mean of 5 levels of coliform content										
0·65	0·76	0·74	0·72	0·71	0·53	0·55	0·42	—	—	0·77
4·40	**4·08**	**3·90**	**3·43**	**3·66**	**2·82**	**2·48**	**2·85**	—	**2·85**	**4·22**
14·7	*18·5*	*19·0*	*21·0*	*19·3*	*18·6*	*22·2*	*14·9*	—	—	*18·3*
Mean of 12 levels of logarithm count										
1·422	1·200	0·816	0·458	0·227	—	—	—	—	—	1·174
−0·58	**−0·15**	**0·46**	**0·86**	**0·78**	**1·0**	**1·0**	**1·0**	—	**1·0**	**−0·13**
245·2	*800·0*	*177·4*	*53·3*	*29·1*	—	—	—	—	—	*903·1*
0·38	**0·71**	**2·88**	**7·24**	**6·03**	**10·0**	**10·0**	**10·0**	—	**10·0**	**0·74**

Main tables 5381	Remarks
1·130 **5·25** *21·5* *(21·4)* **(63·3)**	σ falls as logarithm increases, but v does not alter appreciably (cf. Section 2*a*)
0·77 **3·65** *21·1*	Few samples at extremes of keeping quality; σ and v are greatest at mid-values, 4–8 half-days (cf. Section 3*a*) 4500 per ml. might be 760–26,000 per ml. (to include 66⅔% of result)
5381	
1·131 **4·79** *23·6*	σ improves as coliform increases (cf. Section 1*a*)
1·174 **0·00** *117·4* **1·00**	Most samples 4–6 half-days. v is lower at either extreme (cf. Section 3*b*) +1 ml. might be "abs." or +1/10 ml. (to include 66⅔% of result)
5381	
0·77 **4·22** *18·3*	See remarks, Section 2*a*
1·174 **−0·13** *903·1* **0·74**	σ increases from 0·4 to 1·4 as logarithm increases (cf. Section 2*b*)

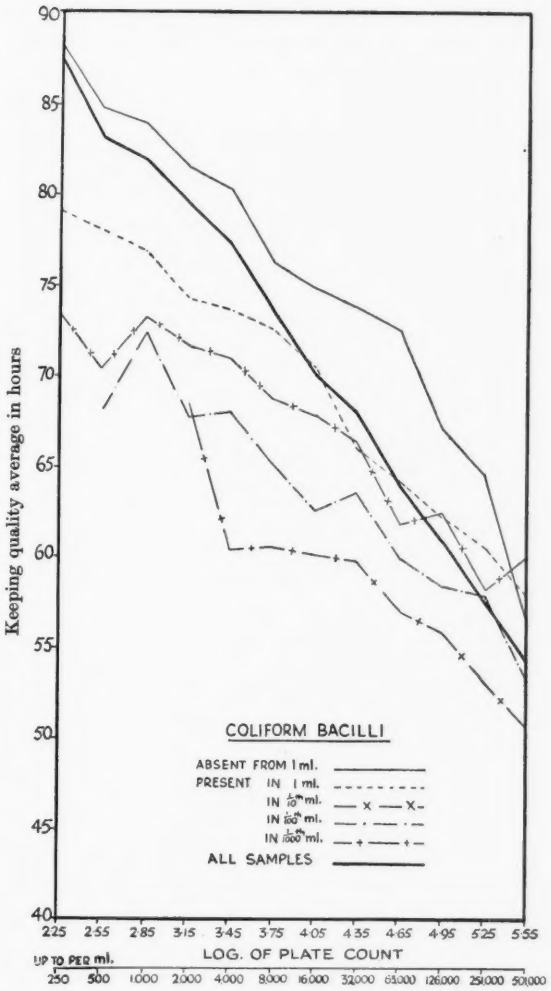

Chart 1. Morning milk.

By courtesy of the *Journal of the South Eastern Agricultural College.*

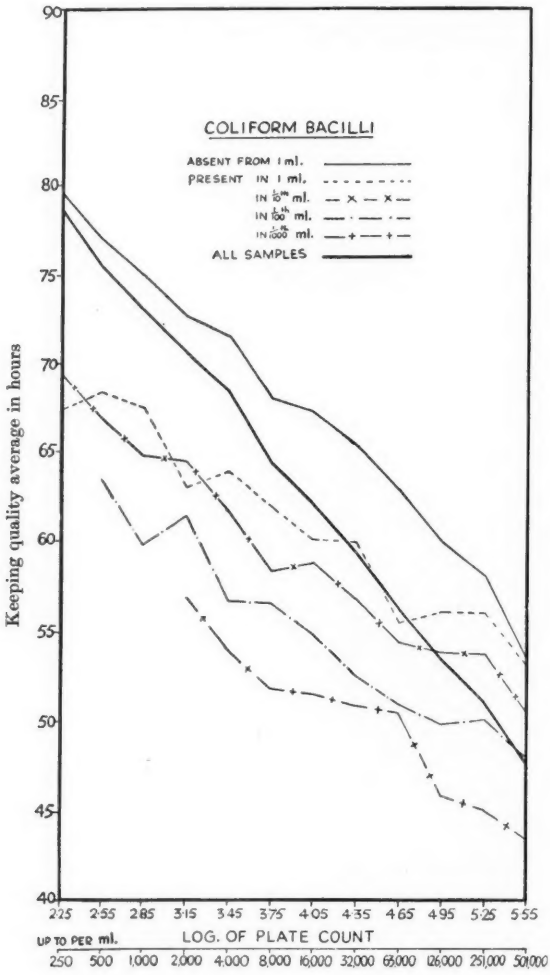

Chart 2. Afternoon milk.

By courtesy of the *Journal of the South Eastern Agricultural College.*

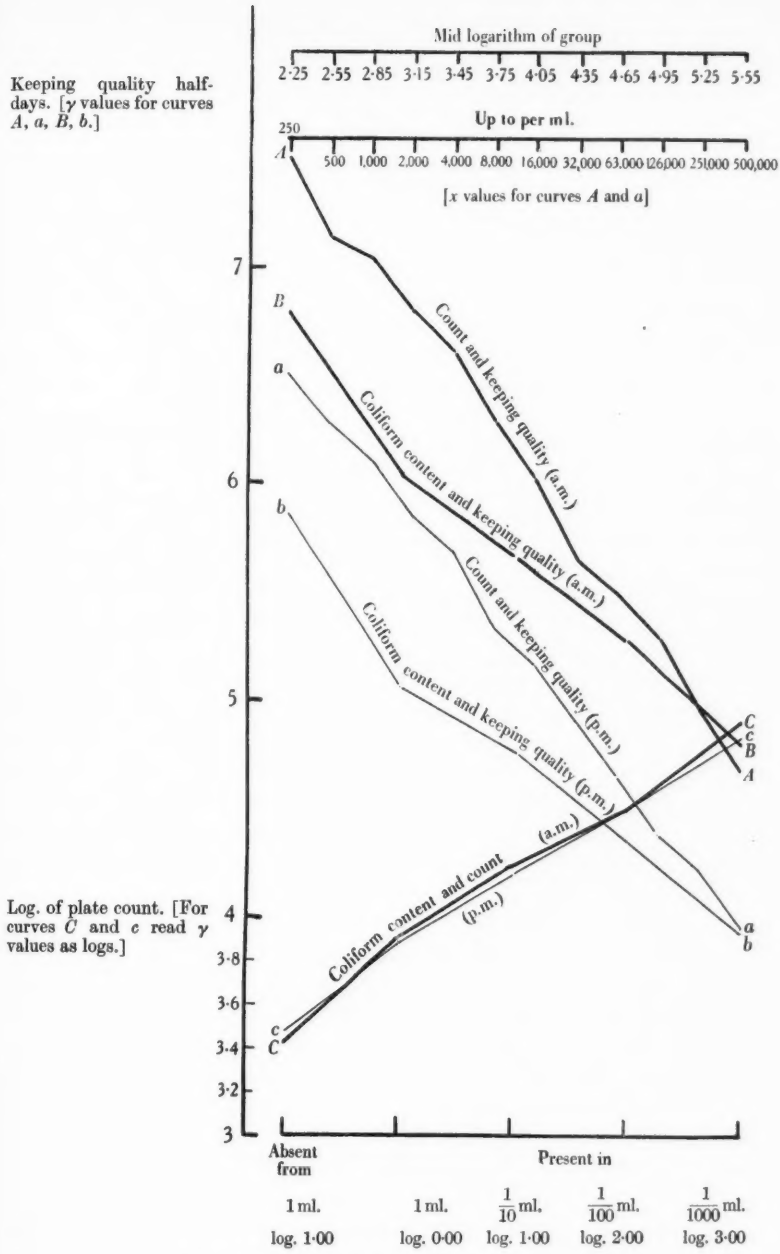

Chart 3. Morning and afternoon milks (see Table V).

Curves *A* and *a*, regression of keeping quality on log. of plate count.
B and *b*, " " " coliform content.
C and *c*, " log. of plate count on log. of coliform content.

Tables VI and VII, for the three main tables, and also for all subtables, while the main variabilities are summarised in Table VIII.

Statistical treatment.

Deviations. The standard deviation was calculated from the usual formula, but it will be realised that each of the standard deviations quoted in Tables VI and VII, for both main and subtables, is the *mean* of the standard deviation of x on y (or y on x) *at all the different levels* of y (or x). This mean of the standard deviations has been calculated from the formula:

$$\text{Mean of the standard deviations} = \sqrt{\frac{S\,[\sigma_p^{\,2}\,(n_p - 1)]}{S\,(n_p - 1)}},$$

where σ_p is the standard deviation of one sample at any particular level and n_p the number of samples at that level, so that the value of the mean of the standard deviations is governed by the value of the standard deviation at the level of maximum incidence of samples and not by the mere number of levels.

Means and variabilities. The means in hours are true means based on the total hours of true life and total samples in each cell, and variability has been calculated from both $\frac{1}{2}$-day and hours data. The hours are grouped on a 12-hour class (see Table II B) and are the same groups as the $\frac{1}{2}$ days, and values of the standard deviations are therefore the same. The $\frac{1}{2}$-day means require no explanation and the logarithmic means for plate count are calculated on the assumption that all samples fall on the mid-logarithm of the class. The class interval for plate count is small, and there can be no serious departure from the true mean. The method of dealing with coliform data has been explained above, and in Tables VI and VII the logarithmic means have been translated into true millilitres, but the variability must be calculated on the logarithmic value.

The standard deviation gives a measure of variability in terms of the variate itself: if the standard deviation of keeping quality on count is 1·13 half-days then the total range of deviation will be of the order of 1 day, *i.e.* $\pm$1·13 half-days. This is based on a probability of 66$\frac{2}{3}$ per cent., a point which is discussed later (p. 46). When comparing variabilities from different series, as is here done, with differing values of the mean it is sometimes preferable to use the *Coefficient of Variability*, which is the standard deviation expressed as a percentage of the mean:

$$\text{Coefficient of variability } (v) = \frac{100 \times \text{standard deviation}}{\text{mean}}.$$

Values of (v) will be found for all main and sub-tables in Tables VI and VII.

Linearity. Fisher(13) has given a method for testing the straightness of regression lines, which is an application of the analysis of variance method to this purpose. The variance is apportioned as that which is within arrays and that which is due to deviations from the regression. The variance due to

deviations from the regression is compared with the variance within arrays. If the former is significantly greater, the regression is non-linear. To test the difference between these two variances a statistical test known as the "Z" test is used. Values of z (equal to half the natural logarithm of the former variance less half the natural logarithm of the latter) have been tabulated for two different levels of significance, namely values which would only be exceeded by chance once in twenty times and once in one hundred times respectively. The value of Z obtained from the data is then compared with the 5 per cent. or 1 per cent. z value obtained from the table, according to the stringency of the criterion adopted. If Z exceeds its 5 per cent. value it is then regarded as showing a significant difference, if greater caution is desired the 1 per cent. value may be used instead. When required interpolated values of z were calculated from the formula given by Fisher ((13), p. 212).

RELATIONSHIP.

General (Tables III and IV). The general interrelationship of the three factors is here disclosed. The mean keeping quality falls as the plate count increases and falls also with increasing coliform content, while a survey of the number of samples in each cell discloses that coliform content also increases with the plate count. Further, a comparison of the mean keeping qualities in Table III with those in Table IV reveals the lower level of afternoon values referred to earlier on. These tables are seen in Charts 1 and 2.

Logarithm of plate count and keeping quality. The correlation is negative and the regression of keeping quality on logarithm of plate count definitely linear. For the main morning table the value of Z is only 0·06, and similar values occur in four out of the five subtables. In the afternoon table the deviations within the arrays are greater than the deviation from the linear except in the subtable for "present in 1/1000 ml." where the value of Z is very small. The weight of evidence is thus all in favour of this relationship being a straight line. The one exception is the group of 753 morning samples giving "present in 1 ml." where Z has a value of 0·4561, while the 1 per cent. value of z is 0·4298. The correlation values are $r = -0{\cdot}5446$ for morning samples and $-0{\cdot}5061$ for afternoon samples. The finding of linearity is in agreement with Robertson and Frayer (4), who examined both linear and non-linear relationships for 353 samples ranging in count from log. 2 to log. 9, held at 60° F. and tasted every 2 hours. These authors state: "there is an unusual high degree of direct or straight line relationship between the plate counts and the keeping quality."

Logarithm of presumptive coliform content and keeping quality. The correlation is negative and the regression of keeping quality on the logarithm of the coliform content non-linear. Values of r calculated from the three linear stations (present in 1, 1/10 and 1/100 ml.) are $-0{\cdot}3087$ and $-0{\cdot}3217$ for a.m. and p.m. Though only one or two of the subtables, dealing with the state of affairs at special levels of plate count, give significant values of Z, the departure

from the linear in the main tables (for all levels of plate count) is indisputable. The 1 per cent. value of z is 0·6656 and the 5 per cent. value is 0·4790, while the values of Z are 1·96 for morning samples and 0·64 for afternoon samples. No indication of non-linearity is found if the last four sections (present in 1, 1/10, 1/100 and 1/1000 ml.) be considered alone or even the inside three (present in 1, 1/10 and 1/100 ml.). The curvature is due entirely to the first group of samples, absent from 1 ml. The explanation would appear to be that the first group is not, as has been assumed for classification purposes, a definite class. Although considered as "absent from 1 ml.," *i.e.* "present in 10 ml.," there are probably many samples "absent from 10 ml." and even "absent from 100 ml." A similar indefiniteness must be admitted for the "present in 1/1000 ml." class.

It would be expected that the relationship between the logarithm of the coliform content and keeping quality would be linear, and this is found in the middle sections (present in 1, 1/10 and 1/100 ml.), where we are dealing with truly definite classes. Failure to disclose curvature in the "present in 1/1000 ml." section suggests that this class contains only a few samples positive in excess of this amount, but the first group must obviously embrace many samples superior in quality to their class group. By calculating a regression line (keeping quality on logarithm of coliform content) from the three definite classes (present in 1, 1/10 and 1/100 ml.) it can be shown that the theoretical values for the "absent from 1 ml." class are 6·5 half-days and 5·54 half-days respectively for morning and afternoon milks. Thus in the case of the first class, "absent from 1 ml.," the observed values, in other words actual practice at the farm, give an average keeping quality which is 0·3 half-days better than the theoretical value. Conversely, on the same regression, the observed values for keeping quality, 6·8 half-days and 5·86 half-days, would be given by milks of coliform content of 1·68 and 1·78 on the logarithmic scale, instead of the class value of 1 on the logarithmic scale. On this basis the average coliform content of this group is not as classified "absent from 1 ml." (which can also be expressed as "present in 10 ml."), but, according to our argument, present in 48 ml. in the case of morning milk and in 60 ml. in the case of afternoon milk.

In other words practical achievement has exceeded the routine testing and the average sample classed as "absent from 1 ml." is really something better both in keeping quality and coliform content.

Logarithm of presumptive coliform content and logarithm of plate count. The correlation is positive and the regression of logarithmic plate count on logarithm of coliform content non-linear. Values of r calculated from the three internal stations (present in 1, 1/10 and 1/100 ml.) are + 0·3444 and +0·3299 for morning and afternoon milks. In the table dealing with all morning samples the 1 per cent. value of z is 0·6656, whereas $Z = 0·95$, and if we omit the last group (present in 1/1000 ml.) the 1 per cent. value of z becomes 0·7641 and the value of Z is 1·1095. There is no significant non-linearity if the first group

be omitted and the last four considered together. The data for afternoon milk are similar, but there is no significant departure from linearity. In the table dealing with all samples the 5 per cent. value of z is 0·4790, and when omitting the last group is 0·5489, while the corresponding values for Z are 0·3865 and 0·4841.

The same arguments apply as in the previous relationship of coliform content and keeping quality, but on comparing the observed values with those calculated from a regression based on the three definite classes of coliform content we find that in the present case of coliform content and plate count the difference between the observed and the theoretical is not so noticeable. In the case of morning milk the observed value (logarithm of plate count) for the first station corresponds to a plate count of approximately 6200 per ml., whereas the value from the regression is approximately 7000 per ml. The difference is even less in the case of afternoon milk.

It must be remembered that these samples were taken during clean milk competitions when producers would be likely to excel the ordinary daily standard of cleanliness. Granted that in almost all the logarithmic count groups the "absent" samples are in the majority, it seems that on the whole a certain amount of coliform contamination is inevitable as the total increases.

Comparison of morning and afternoon milks.

Significance. In the relationships in which coliform content is concerned it will be noted that the significant figures discussed above are always less definite for afternoon milk than for morning milk, but Table VIII will show that the standard deviation for coliform content for afternoon milk is greater.

Means. Table V shows that when keeping quality is considered there is a clear difference between the mean values of keeping quality for morning and afternoon milks at corresponding points, whether we deal with the effect of plate count or of coliform content. The irregularities of the keeping-quality test may be a cause, especially in the case of afternoon milk at the morning tasting, and the difference might thus be at least in part more apparent than real. Barkworth (7) has shown that the difference when dealing with morning and afternoon milks of similar plate count is numerically lessened by dealing in terms of true life hours.

The standard deviation is the same by hours or ½ days because the grouping is identical, but changes occur in the coefficient of variability because the means in hours for corresponding groups are lower, *e.g.* the mean for ½-day group for morning milk is 11 hours and for afternoon milk 13½ hours. Total fall in keeping quality is the same in both sections of Table V (*a*) and (*b*), but the fall in means of Table V (*b*) shows that coliform content has a marked effect on keeping quality. In matters of coliform content and plate count, however, morning and afternoon samples are on an equal footing. Table V (*c*) and the slightly higher standard deviation of coliform content would appear insufficient to account for the comparatively lower values for afternoon milk in Table V (*a*).

It follows from Table V (*a*) and (*b*) that where mean keeping-quality values are under consideration, results for morning and afternoon milks must be tabulated separately, and the columns of Tables III and IV dealing with coliform organisms show that even the separated means of Table V (*a*) do not tell the whole story because coliform content also affects keeping quality. On the other hand there is a direct relationship between logarithmic plate count and logarithmic coliform content, and in this relationship morning and afternoon milks are on an equal footing.

Because of this relationship between plate count and coliform content it may be claimed that although the results in Tables III and IV for the "absent from 1 ml." class may be regarded as the keeping quality attainable at a given plate count, nevertheless the true average keeping quality at a given plate count is as shown in Table V (*a*), where the results are governed by both the level of the plate count and the average coliform content at that particular level of plate count.

Regression values.

From the regression coefficients derivable from the data of the three mean tables as defined on page 33 we can obtain the following equations:

Morning samples: $$(y-\bar{y})=\frac{(x-\bar{x})}{-0{\cdot}80},$$

$$(y-\bar{y})=\frac{(x'-\bar{x}')}{0{\cdot}43},$$

where y = keeping quality,

x = logarithmic plate count,

x' = logarithmic coliform content.

Now $y-\bar{y}$ is identical in both equations. This is because the distribution *as regards keeping quality* must be the same, although of course the distribution will not be the same in regard to logarithmic plate count in twelve groups, as it is in the case of logarithmic coliform content in five groups.

Because the left-hand side of equations (*a*) and (*b*) is the same we can write:

$$\frac{(x'-\bar{x}')}{0{\cdot}43}=\frac{(x-\bar{x})}{-0{\cdot}80},$$

$$\therefore \quad (x'-\bar{x}')=-0{\cdot}54\,(x-\bar{x}).$$

A similar calculation can be made for afternoon milk where the coefficients are $-0{\cdot}70$ and $-0{\cdot}40$, giving a factor of 0·57.

It follows that an increase of 0·54 in the logarithm of plate count in the case of morning milk or 0·57 in the case of afternoon milk, is equal to a decrease of one class interval, e.g. from 1/10 to 1/100 etc., in the coliform test in regard to the decrease of keeping quality which it produces.

This means that an increase of one stage in the presumptive coliform test decreases the average keeping quality as much as a sevenfold increase in count.

Variability.

Sources of error. The variabilities are shown in Tables VI and VII and summarised in Table VIII. The standard deviation of the main tables is larger than the standard deviation of the subtables (except in a few cases) and is always greater than the mean of the standard deviations of the subtables. The fluctuations in value are the gross effect of several causes—technique, errors of the test and the true variability. The technique followed has been noted. Mattick (14) and Mattick, Hiscox and Christian (15) have discussed the factors affecting the plate count, and a note on media will be made later. Chalmers (16), Edmunds (17), Kon (18), and Malcolm (19) have discussed the different organisms reacting as coliform organisms in milk, and Malcolm (*loc. cit.*) states that under winter conditions 71 per cent. of the strains were of true *Bact. coli* types. Hammer (20) states that the different species of the group vary in the effect of temperature on their activity, and Hammer and Yale (21) have shown that at least in butter the *aerogenes* type is the more deleterious at 18° C. It does not appear that any work has been done on the effect of different strains of the *coli-aerogenes* group in the rate of deterioration of milk. Some error will arise due to the use of single tubes at each dilution, but should be minimised by the grouping system employed. If *coli-aerogenes* organisms have a particularly deleterious action on milk it should be noted that while presumptive coliform tests are made at 37° C. the keeping quality test is made at 15·6° C., and not only will this affect the proportionate growth of different species of the *coli-aerogenes* group, but some members of the *aerogenes* section will not ferment lactose at 37° C. as shown by Grimes and Hennerty (22) in strain from butter, and Hiscox (23), strain from cheese milk. In addition to the difficulties of interpreting keeping-quality tests the test itself would be subject to error, such as the error due to different workers tasting the same sample and due to one worker tasting duplicate samples of the same milk. The tasting has throughout been done by two people, the writer and his assistant, and close co-operation has been possible. The writer has no knowledge of any work attempting to measure the two sources of error just named. Hiscox *et al.* (24) have suggested that the keeping-quality test may be replaced by the methylene blue reductase test. This would eliminate personal error. Finally a reference must be made to media. All plate counts were made on a simple lemco-peptone agar, but Hiscox *et al.* (24) have shown that this medium may fail to grow many organisms, and the use of a simple agar according to the prevailing standard technique of the period undoubtedly contributes to variation of keeping quality in terms of plate count.

Variability. Although differences occur in the standard deviations and to greater extent in the coefficients of variability (due to altered level of the mean) these measures of variability are on the whole similar, and it would seem that if variability alone is considered, morning and afternoon milks might be combined. This would, however, be misleading if we wish to establish mean keeping-quality values at any particular level of plate count. The lowest value

of standard deviation of keeping quality is 0·82 half-day for "present in 1/1000 ml." for afternoon samples, while the highest figure is 1·22 half-days for morning samples when dealing with variation of keeping quality on coliform content, and although the variability on plate count is improved by differentiation for coliform content it is not seriously reduced, and it seems that a standard deviation of the order of 1 half-day is the limit of accuracy of the present test. According to the standard deviation (or coefficient of variability) the probable keeping quality can be forecast within the same margin of error from either coliform content or logarithmic plate count, so that coliform content is as important as organisms shown by the plate count in determining the keeping quality. Having established the variability we can consider the margin which must be exceeded before we can consider the keeping quality of a sample to be abnormal. If we wish to include 95 per cent. of the results then the margin would be ± 2 half-days, *i.e.* $\pm 2\sigma$. If we accept the easier standard of $66\frac{2}{3}$ per cent. the margin is only ± 1 half-day ($\pm \sigma$), which is the same result as in previous work by the writer(9) based on the same standard of accuracy. The increasing use of the keeping-quality test has led to much injudicious criticism through failure to realise this large normal variability, while the layman is prone to query the slightest fall below expectation. Before leaving the subject it may be pointed out that marks for keeping quality have nothing to do with the test. The keeping-quality test is frequently used in conjunction with the plate count and/or other tests and samples may be graded according to various marks scales. The marks scale for keeping quality may be faulty in itself or out of balance with marks for other tests, but obviously such errors must not be blamed on the keeping-quality test or the method of interpreting and recording results. At the same time data here presented should enable suitably balanced scales of marks to be devised for all three variates.

Turning to the reverse of the picture we find that the variability is even less practical, and the variability of plate count is 0·77 on the logarithmic scale, being substantially the same from all data, coliform content or keeping quality, morning or afternoon samples. Thus if a sample were classified by coliform content and keeping-quality test, and the plate test omitted we could predict its probable plate count with a standard error of 0·77 on the logarithmic scale. On a standard to include 95 per cent. of the results this would mean that the plate count could increase or decrease about 35 times for the same results, and on a $66\frac{2}{3}$ per cent. basis the possible increase or decrease would be 4–6 times. If plate count is to be forecast the coefficient of variability would have to be reduced to $2\frac{1}{2}$ per cent. to meet the requirements of the 95 per cent. standard and to 5–10 per cent. to meet the $66\frac{2}{3}$ per cent. standard, instead of approximately 20 per cent. as found from the tables. The standard deviation of the logarithm of the coliform content is also very high, being over unity, and as the class interval is unity even on a $66\frac{2}{3}$ per cent. basis the possible increase or decrease would involve three classes. In other words

if we try to forecast coliform content from either plate count or keeping quality an expectation of "present in 1/10 ml." might in fact be "present in 1 ml." or "present in 1/100 ml."

While a measure of variability under existing technique is thus established, it is plain that among the three variates—plate count, coliform content and keeping quality—the variability is too great for practical purposes to admit of forecasting any term from results of the other two.

SUMMARY.

The writer seeks to establish the interrelationship and variability of the three tests used for gauging the hygienic quality of milk, plate count, coliform content and keeping quality. Keeping quality is defined, and after discussing previous work and source of material, and noting the technique followed, the method of classification is described. Plate count and coliform content are treated logarithmically. Treatment of keeping-quality results presents especial difficulties, and the test is described in detail to disclose these, and reasons given for tabulating by half-days. The relationship between plate count and keeping quality is linear, but non-linearity occurs in those relationships which concern coliform content. This is because the first group "absent from 1 ml." is not truly a definite class, but contains samples of superior quality. It is shown that *an increase of one "stage" of coliform contamination reduces the average keeping quality as much as an increase of* 0·54 *in the logarithm of the plate count* (*approximately seven times*). Variability of each term is discussed. The standard deviation of keeping quality is of the order of 1 half-day, that of logarithm of plate count is 0·77 and that of logarithm of coliform content in excess of unity. These variabilities are too great to permit of reasonable forecast of one term from the other two.

ACKNOWLEDGMENTS.

The writer gratefully acknowledges his indebtedness to Mr T. N. Hoblyn of the East Malling Research Station, for advice and guidance in statistical treatment. Assistance has also been received from Mr H. B. Bescoby of the South-Eastern Agricultural College, Wye, Kent.

REFERENCES.

(1) HOY (1922). Private correspondence.
(2) BARKWORTH, MATTICK, TAYLOR and WILLIAMS (1927). *J. Minist. Agric.* **33**, 997–1001.
(3) HOY and NEWLAND (1931). *Proc. Soc. agric. Bact., Rep. Ann. Conf.*
(4) ROBERTSON and FRAYER (1930). *Vt agric. Exp. Sta. Bull.* Nos. 314–18.
(5) BARKWORTH (1930). *J. Minist. Agric.* **37**, 803–6.
(6) THOMAS and JONES (1931). *Welsh J. Agric.* **7**, 304–10.
(7) BARKWORTH (1931). *J. S.-E. agric. Coll. Wye*, **28**, 270–2.
(8) —— (1931). *J. Hyg.* **31**, 373–4.
(9) —— (1931). *J. S.-E. agric. Coll. Wye*, **28**, 273–7.

(10) *Misc. Publ. Minist. Agric.* No. 43, 1st ed. 1924, 2nd ed. 1926, 3rd ed. 1928.
(11) BARKWORTH (1929). *Sanit. (Insp.) J.* **34**, 9, N.S. 270–1.
(12) —— (1933). *J. S.-E. agric. Coll. Wye*, **32**, 189–93.
(13) FISHER (1932). *Statistical Methods for Research Workers.* 4th ed. pp. 229–35.
(14) MATTICK (1930). *J. Dairy Res.* **1**, 111–34.
(15) MATTICK, HISCOX and CHRISTIAN (1933). *Ibid.* **4**, 285–8.
(16) CHALMERS (1928). *J. Hyg.* **27**, 295–305.
(17) EDMUNDS (1932). *Minist. Agric. Ann. Meeting Dairy Bacteriologists.*
(18) KON (1933). *J. Dairy Res.* **4**, 206–12.
(19) MALCOLM (1933). *Ibid.* **5**, 15–28.
(20) HAMMER (1928). *Dairy Bacteriology*, p. 41.
(21) HAMMER and YALE (1932). *J. Dairy Sci.* **15**, 199–208.
(22) GRIMES and HENNERTY (1931). *Sci. Proc. R. Dublin Soc.* **20**, 89–97.
(23) HISCOX (1932). *Min. Agric. Ann. Meeting Dairy Bacteriologists.*
(24) HISCOX, HOY, LOMAX and MATTICK (1932). *J. Dairy Res.* **4**, 105–21.

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92. AN AEROBIC ACID-FAST *ACTINOMYCES* IN COW'S MILK DUE TO AN UDDER INFECTION

By J. D. ALLAN GRAY.

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(With 3 Figures.)

THE isolation from cow's milk of an organism allied to *Actinomyces farcinicus* merits recording on account of the rarity in this country of such organisms and on account of failure to find in the literature a description of the organism.

CLINICAL HISTORY OF THE COW.

(Communicated by Mr E. C. BOVETT[1], M.R.C.V.S.)

A Guernsey cow three years old was imported from Guernsey in May 1930 and placed in a grade A herd. At the regular quarterly examinations neither she nor other members of the herd showed evidence of udder or other disease before she became ill. She had calved four times since her arrival in 1930, the last two calves being born on January 11, 1933, and January 7, 1934. After the last calving one of the fore-quarters of the udder became acutely inflamed and for about a fortnight there was a considerable general reaction with a temperature of between 106° and 107°. The calf sucked for the first two days before transference to another animal. It is not known whether it obtained milk from the affected quarter, but up till now it has remained well. Gradually the cow's general condition improved, but an abscess developed in the affected udder and burst on April 6, setting free a quantity of purulent material. Since then the cow had gained in weight and by the middle of April was giving $2\frac{1}{2}$ gallons of milk per day from the three sound quarters. The affected quarter was amputated on March 16 and the wound healed by first intention.

Bacteriology.

The first sample of milk (February 3, 1934) was submitted for examination for the presence of tubercle bacilli (and so unfortunately cultures were not made). The centrifuged deposit contained pus cells, but neither tubercle bacilli nor *Actinomyces* were found. The guinea-pig inoculated developed in 18 days a large localised abscess. The pus of this abscess contained numerous beaded and branching filaments and from it was grown a pure culture of the *Actinomyces* described below.

[1] The writer has to thank Mr G. E. Henson, M.R.C.V.S., and Mr E. C. Bovett for the clinical details and material.

The second and third samples of milk (February 24 and March 12, 1934) both microscopically showed staphylococci but not *Actinomyces*. Culture and guinea-pig inoculation, however, revealed the presence of the *Actinomyces* in both samples.

The affected quarter was amputated on March 16, 1934. It was greatly enlarged (weighing 3500 gm.), reddened and possessed a disagreeable sour odour. The abscess cavity communicated with the exterior by a sinus opening about 3 in. above the teat. Histologically there was a slight fibrosis and both staphylococci and *Actinomyces* were seen. The presence of both organisms was confirmed by culture and guinea-pig inoculation.

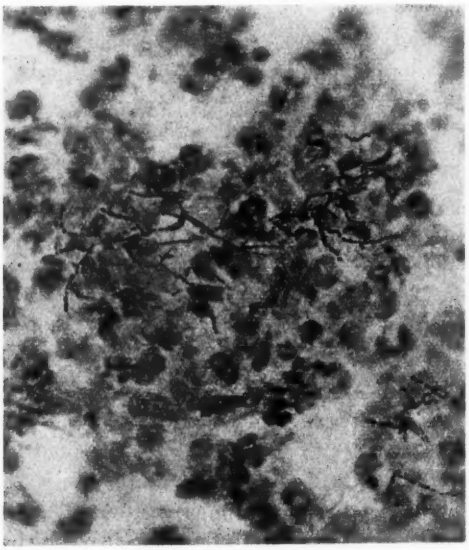

Fig. 1. Beaded filaments in pus from subcutaneous abscess from guinea-pig.

Description of the Actinomyces.

The strains obtained from the guinea-pigs inoculated with the deposits of the milk samples and udder emulsion were identical in the characteristics investigated.

Morphology.

Long branching filaments showing dichotomous branching. The majority are markedly beaded and frequently present a streptobacillary or streptococcal appearance with a trace of membrane uniting the fragments (Fig. 1). Mycelial formation is greater in pus than in culture but never marked except in the

kidneys of the rabbit (*vide infra*). Cultural forms, especially those in fluid cultures aged a fortnight or more, frequently show complete fragmentation into bacillary or coccal forms. Conidia and clubs similar to those seen in *Actinomyces bovis* are not observed even in sections of animal lesions.

Staining reactions.

The beaded filaments and the bacillary and coccal forms are invariably Gram-positive. Occasionally in old cultures Gram-negative filaments are found. These never show beading or fragmentation.

The organisms are decolorised by the 20 per cent. aqueous sulphuric acid or 3 per cent. hydrochloric acid in alcohol of the usual Ziehl-Neelsen method. When, however, 1 per cent. aqueous sulphuric acid is used for decolorising, the source and age of the organisms considerably influence their reaction. Filaments from young cultures are typically not acid-fast, but in young fluid cultures a few acid-fast coccal forms are found both on the floating mass and to a lesser extent in the deposit. Incubation for several weeks produces a marked relative increase in the acid-fast elements, especially in the deposits.

Culture.

The organism is a strict aerobe and grows well on ordinary media at temperatures between 23° and 40° C. After 24 hours the colonies on agar are small, dry, irregular, greyish white and raised and moderately firmly adherent to the surface of the medium. After about a fortnight the raised centre becomes a bright orange and the colony grows to about a centimetre in diameter with irregular outline (Fig. 2). When confluent, the growth has a rough wrinkled buff surface.

In broth grey masses develop on the surface. On shaking most of these fall to the bottom of the flask.

Gelatin is not liquefied. A faint growth appears very slowly along the line of inoculation, and after about 9 weeks at room temperature develops a bright orange colour.

On potato there develops a dry, scaly, orange-yellow growth. Litmus milk is not altered and nitrates are not reduced. Acid and gas are not produced in tubes of peptone water containing glucose, lactose, saccharose, dulcite, maltose and mannite. The organism grows well in Koser's citrate.

Resistance.

When grown in sterile milk for 8 days the organism survives an exposure to the temperature of pasteurisation (145° F.) for 1 hour but is killed in 5 min. by 100° C.

Pathogenicity.

Guinea-pigs and rabbits are susceptible, the former to subcutaneous, intraperitoneal and intravenous inoculation and the latter to intravenous inoculation only. Rats and mice are refractory. All feeding experiments of guinea-pigs, rabbits, rats and mice failed to produce infection.

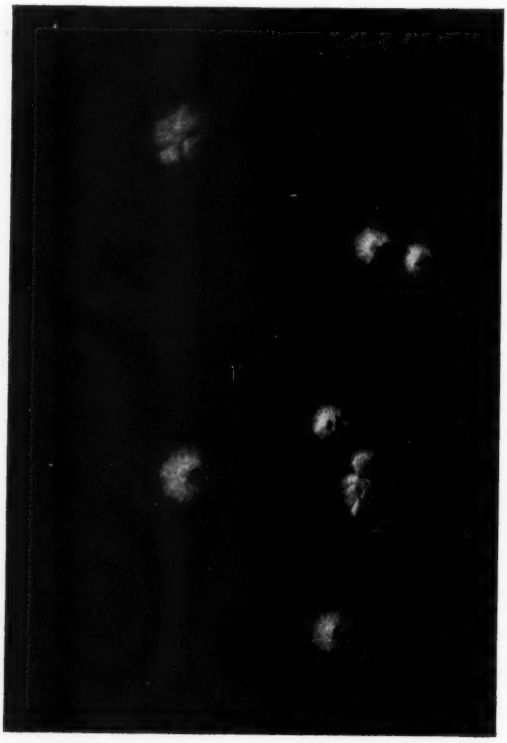

Fig. 2. *Actinomyces* culture on agar after incubation for 7 days at 37° C.

Subcutaneous inoculation of guinea-pigs produces a local abscess which usually bursts and heals of its own accord. Reinfection is possible. The pus from such an abscess on repeated washing with saline is found to contain sticky masses visible to the naked eye. These masses contain numerous filaments and are presumably analogous to the "sulphur granules" in the pus of actinomycosis due to *A. bovis*. At first, abscesses are produced in about 3 weeks after inoculation, but later, after passage through several guinea-pigs, the incubation period is reduced to about 5 days.

Intraperitoneal and intravenous inoculation of guinea-pigs is fatal in about

1–3 weeks, miliary nodules appearing on the peritoneal, pericardial and pleural surfaces (Fig. 3). These nodules contain pus cells and numerous filaments.

Intravenous inoculation of rabbits produces a fatal septicaemia in 2 days, miliary nodules being found in the internal organs, and microscopically the filaments are found in very large numbers in the internal organs and especially in the glomerules of the kidney.

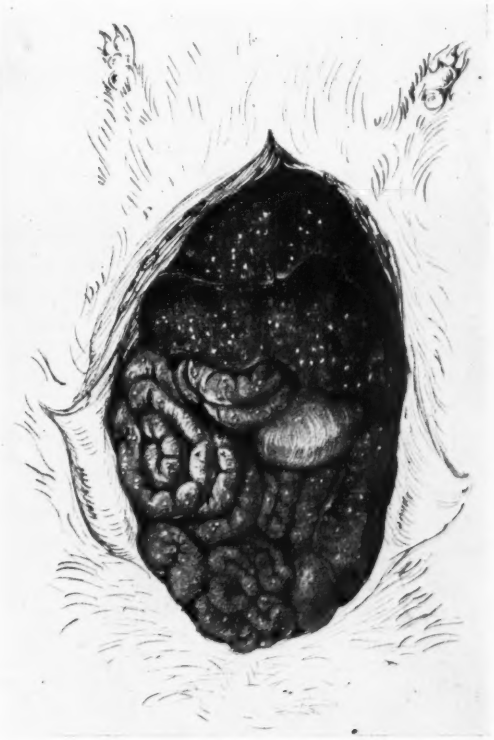

Fig. 3. Miliary nodules on serous surfaces in a guinea-pig.

Discussion.

In the accounts of numerous *Actinomyces* which have been recorded, many of the forms, while not identical, have borne marked resemblances to each other. Not only is the determination of different types therefore difficult, but it is almost impossible to be certain that a newly isolated *Actinomyces* has not been previously described. Considerable importance naturally attaches from the bacteriological standpoint to the description of any new species, but it is a moot question whether certain of the forms which have been recorded warrant description as species hitherto unknown.

A further factor which has added to the difficulties in the identification of

Actinomyces is the suggestion of Wright(1) that many of the saprophytic (aerobic) forms common on grains and grasses are liable to gain access as contaminants to lesions caused by other *Actinomyces* and may have erroneously been regarded as the aetiological agents of the lesions concerned. It was for this reason that anaerobic cultures were made from the milk deposits and udder emulsion. These consistently failed to demonstrate the presence of anaerobic organisms. It is therefore believed that the organism described above was the aetiological agent of the udder disease. It does not conform exactly to any of the species which have been described in the literature investigated, but apart from its pathogenicity for the rabbit it is closely allied to *A. farcinicus* originally isolated by Nocard(2) from cattle suffering from bovine farcy.

The occurrence of *Actinomyces* in milk has been noted by several observers. Those studied by Berestnew(3) and Chalmers(4) are presumably of the saprophytic type and not of importance in human and animal pathology. Chalmers found their occurrence to be comparatively rare (about 5 per cent. of the samples examined) and slightly commoner in winter than in summer. Their growth in milk even at 25° C. was slow, and they were all thermostable to the extent of resisting an exposure to 60° C. for 30 min.

A streptothrix infection of cow's udders was recorded by Evans(5), for which an organism similar to the one here described was responsible. It was, however, comparatively thermolabile in being killed by a temperature of 56° C. for 30 min. The results of animal inoculation are not given.

Of other aerobic acid-fast *Actinomyces* which are of importance in human and animal pathology, *A. asteroides* (Eppinger(6)) differs from this organism by producing stellate colonies on agar and a red pigment on potato; *A. gypsoides* (Henrici and Gardner(7)) by liquefying gelatin, and another strain by digesting milk (Birt and Leishman(8)).

The origin and avenue of infection in this case are unknown. As far as can be ascertained, the cow did not receive injury to the affected quarter, and it is suggested that the organism originally in fodder or bedding gained access via the teat (see Wall(9)). No other lesion could be found in the cow. Had the quarter affected not been a fore one, one might have suspected that the udder disease was a metastatic lesion.

The hygienic importance of the presence of the *Actinomyces* in cow's milk must be a matter for conjecture, as it is not known whether the particular strain is pathogenic for humans. Numerous human infections with aerobic acid-fast *Actinomyces* have been recorded (Eppinger(6), Birt and Leishman(8), MacCallum(10), Namyslowski(11), Cohn(12), Henrici and Gardner(7)). On the other hand certain aerobic types have been considered to have no relation to any disease present (Berestnew(3), Lord(13), Thjøtta and Gundersen(14) and Gerbasi(15)). In this connection, the negative results of the writer's animal feeding experiments are of interest. The ability of cultural forms of the organisms in milk to resist a temperature of 145° F. for 1 hour suggests that

pasteurisation of milk could not eliminate any risk which its presence in the milk might entail.

This investigation has been considerably facilitated by a grant from the Colston Research Grants Committee.

REFERENCES.

(1) WRIGHT (1905). *J. med. Res.* **13**, 349.
(2) NOCARD (1888). *Ann. Inst. Pasteur*, **2**, 293.
(3) BERESTNEW (1898). Quoted by Fiestmantel (1902). *Zbl. Bakt.* I, Orig. **31**, 433.
(4) CHALMERS (1930). *Proc. Soc. agric. Bact.* (Private circulation through the courtesy of Staff of National Institute for Research in Dairying, Shinfield, Reading.)
(5) EVANS (1918). *J. infect. Dis.* **23**, 373.
(6) EPPINGER (1891). *Beitr. path. Anat.* **9**, 287.
(7) HENRICI and GARDNER (1921). *J. infect. Dis.* **28**, 232.
(8) BIRT and LEISHMAN (1902). *J. Hyg.* **2**, 120.
(9) WALL (1918). *Mastitis of the Cow.* Philadelphia: Lippincott and Co.
(10) MACCALLUM (1902). *Zbl. Bakt.* I, Orig. **31**, 529.
(11) NAMYSLOWSKI (1912). *Ibid.* **62**, 564.
(12) COHN (1913). *Ibid.* **70**, 290.
(13) LORD (1910). Quoted by Topley and Wilson (1929). *The Principles of Bacteriology and Immunology.* London.
(14) THJØTTA and GUNDERSEN (1925). *J. Bact.* **10**, 1.
(15) GERBASI (1927). *Zbl. Bakt.* I, Orig. **101**, 369.

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637.16:541.1

93. ON THE DETECTION OF ADDED WATER IN MILK

By BENJAMIN DAVIES.

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(With 6 Figures.)

Test methods previously used.

Hitherto there have been in use three main tests for added water, besides the legal tests for fatty and non-fatty contents, viz.:

(1) Refractive index.
(2) Electrical conductivity.
(3) Freezing-point.

It has been found by observers in general that no certain test for added water is afforded by measurement of any *one* of the above quantities, and this has been confirmed by the present writer in regard to the refractive index and conductivity. This arises from the marked daily and seasonal variations in the values of these physical quantities for milk. The freezing-point was not investigated in the series now under consideration, though it was studied in a preliminary manner before the series began. But a third quantity, viz. the rotation of the plane of polarisation by the serum of the milk, has been studied along with the first two named. This again, by itself, affords no certain test of adulteration.

Accordingly, after studying (in 176 separate tests) the refractive index, conductivity and the rotation of the plane of polarisation of milk, pure and adulterated, over a period of 12 months for a herd averaging eleven cows, an attempt was made to find out if a *combination* of these three sets of observations would lead to a formula which might be taken as a quantitative measure of the essential quality of pure milk from the herd in question; and which quantity, when plotted against the time axis, would smooth out the seasonal variations and give a curve approximately parallel to the time axis, so that any serious departure from that curve would indicate with certainty a definite and measurable degree of adulteration.

Denoting refractive index by μ, conductivity by K, and the angle of rotation of the plane of polarisation by P, various methods of combining μ, K and P were tried with but partial success until there was substituted for μ the quantity $\mu-\mu_0$, where μ_0 is the refractive index of pure water at the temperature of the tests, namely 1·3330 at 20° C. We shall denote $\mu-\mu_0$ defined above by $\Delta\mu$, and use the symbol M to denote the physical characteristics of milk measured by a combination of the values of $\Delta\mu$, P and K.

Out of the many tried, the following are the expressions for M which were found most promising and worthy of further study:

(1) $$M = \alpha\Delta\mu + \beta P + \gamma K,$$

where α, β and γ are constants to be determined by trial.

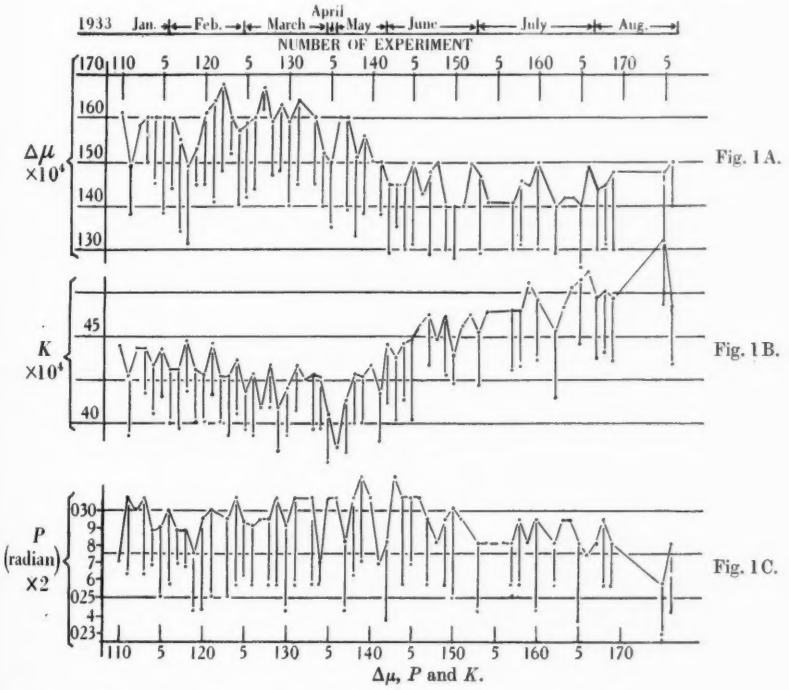
(2) $$M = \Delta\mu . P . K,$$

(3) $$\frac{M_1}{M_2} = \frac{(\Delta\mu PK)_1}{(\Delta\mu PK)_2},$$

being the ratio of the values of M on two successive days for the pure milk.

(4) $$\frac{M_p}{M_w} = \frac{(\Delta\mu PK)_p}{(\Delta\mu PK)_w},$$

where p refers to pure milk and w to the watered milk.



The failure of any one single physical quantity $\Delta\mu$ or P or K to afford a safe test of added water is clearly shown in Figs. 1 A, B, C.

Fig. 1 A shows the variation of refractive index for pure milk over a period of 8 months, and the vertical lines show the effect of 10 per cent. added water. It is clear that the daily variations are comparable with, and at times even exceed, the effect of the 10 per cent. added water, and so rule out the possibility

of a safe test. The same remarks apply to Fig. 1 B and C for conductivity and the rotation of the plane of polarisation respectively.

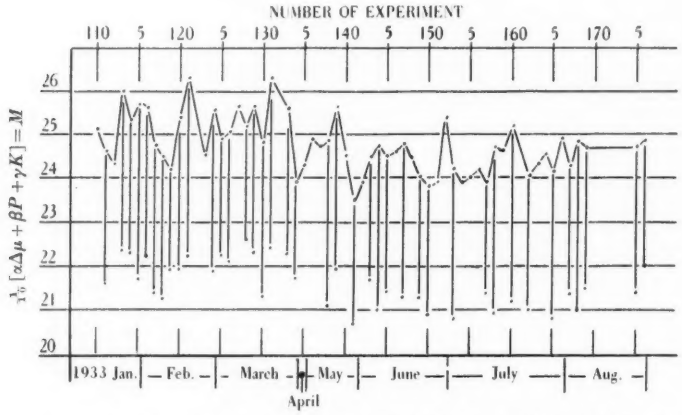


Fig. 2. $\alpha\Delta\mu+\beta P+\gamma K$ for herd of 11 cows. Showing effect of 10 °/₀ added water.

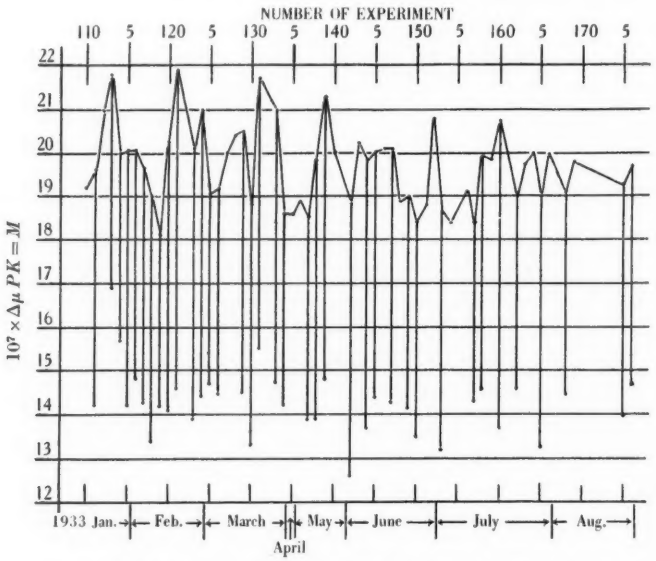


Fig. 3. $\Delta\mu PK$ for herd of 11 cows. Showing effect of 10 °/₀ added water.

On the other hand, Fig. 2 shows the daily variation of the quantity $\alpha\Delta\mu+\beta P+\gamma K$ in which, it will be observed, the effect of added water (again shown by the vertical lines) is decidedly greater than the daily variations. And the same remarks apply with added force to the curve in Fig. 3, which shows the daily variations of the quantity $\Delta\mu PK$.

Furthermore, it is clear that the seasonal variations practically disappear in the last curve. It may, therefore, be claimed that a suitable combination of measurements of refractive index, conductivity and rotation of the plane of polarisation affords, for a given herd, a safe and certain test for added water in milk at any season of the year.

The above remarks apply, as was stated, to a given herd.

It would, however, be a further step in advance if a quantity could be obtained, the measurement of which is independent of the herd, that is, a quantity which would apply equally to all herds.

This idea has not yet been fully explored, for the main part of the work has been limited to a single herd. A few measurements on milk from single cows of different breeds have, however, given results such that the author believes he is on the right lines in assuming that since the quantity $\Delta\mu PK$ gives a quantitative measurement of the quality of pure milk for a given herd, therefore the ratio $(\Delta\mu PK)_1/(\Delta\mu PK)_2$ (where the suffixes refer to measurements made on two successive days) will give a quantity which will not differ by more than a small percentage from unity, provided, of course, the two milks are pure. It is shown further that a safe figure for this possible variation from unity is approximately 8 per cent.—a figure likely to be true for all herds.

APPARATUS AND TECHNIQUE.

In the course of the work the following quantities have been measured: refractive index μ, rotation of plane of polarisation P, electrical conductivity K, and the allied electrical quantities capacitance and the phase angle θ.

Refractive index. For this measurement a Bellingham and Stanley refractometer of the Abbé type was used. As is well known, this depends on the principle of total internal reflection, and the measurement consists in measuring the "critical angle." Under suitable conditions this gives μ to the fourth place of decimals.

At first, considerable difficulty was found in applying this instrument to milk, for within a few seconds of introducing the milk the definition was destroyed. This was ultimately traced to the creaming of the milk. In normal use, the *clear* glass surface of the instrument is uppermost and the *mat* surface undermost. Thus the rapid rise of the cream to the clear surface caused loss of definition. This difficulty was overcome by tilting the instrument so that the clear surface was undermost. The tilting of the instrument revealed another difficulty, viz. a slow deposition of small particles downwards on the clear glass. Fortunately the deposition is so slow a process that as a rule there is a period of some 5 min. at the observer's disposal before the definition is affected.

The temperature of the instrument was kept constant by means of water circulation from the large constant temperature tank in which the electrolytic cell was placed. The temperature of this tank was kept constant to within 0·02° C. throughout the series.

The stirring of the water in the tank was a continuous operation performed by means of compressed air from a small compressor driven by an electric motor. Part of this compressed air was taken through an ordinary glass filter pump which was used inverted, so that instead of the water exhausting air, the compressed air from the compressor was made to circulate water around the refractometer. The stirring of the water in the tank and the circulation of water around the refractometer were thus automatic.

Rotation of the plane of polarisation. To obtain measurable angles of rotation, the path of the light in the liquid must be several centimetres long, and the cell used in these experiments was actually 4 cm. in length. The milk itself could not be utilised owing to its opacity, and the serum obtained by copper precipitation was therefore employed. The source of light was a pointolite lamp. A system of lenses was so arranged between the polariser and the source that the white-hot sphere of the lamp could be seen at the analyser as a sharply defined bright disc. The measurement was made by rotating the analyser till the line of demarcation between the complementary colours of the bluish serum lay along a diameter of the bright disc. The analyser carried a mirror reflecting a beam of light onto a distant scale on which the rotation was read.

The only trouble experienced with this simple polariscope arose from an occasional excessive turbidity of the serum. For most of the readings, however, the rotation was measured with moderate accuracy (within 5 per cent.).

Electrical conductivity. The electrolytic cell was of glass with grey platinum electrodes; the distance between the electrodes was 2·0 cm. and the area of each was 1 sq. cm.

The original intention was to measure the resistance R, the capacitance C and the phase angle θ of the milk, for it is well known that an electrolytic cell acts as if it were a combination of resistance and capacitance in parallel with a certain phase angle between; the combination having a certain impedance, the reciprocal of which was the effective conductivity sought.

It was found, however, that for the particular cell used, the capacitance, though fairly considerable at the outset, fell so seriously on occasions during the course of the work that the phase angle θ became too near 90° to be available for accurate measurement. And so, only the simple conductivity K is of real use in the present series.

The method adopted was the usual Wheatstone Bridge with earphones as a detector. The current used was a fairly pure sine wave alternating current of frequency 540 $\sim$ per sec. The balance was good, giving complete silence in the phones; the limiting accuracy being about 1 part in 500.

Criteria of "utility" for the detection of added water.

A study of the separate curves in Figs. 1 A, B and C, 2 and 3 shows that the quantities measured exhibit marked daily variations, and in all the figures except Fig. 3 ($\Delta\mu PK$) the quantities also show seasonal variations as well.

Let V_d be the daily variation and V_s the seasonal variation. Let M_p be the value of the quantity measured for pure milk and M_w be the same for milk containing 10 per cent. of water.

Now in order to be just to the farmer, the *maximum* values of both V_d and V_s must be taken into account, otherwise he will be liable to prosecution though selling genuinely pure milk.

Thus, the "utility" U of any method of detection that will be just to the farmer is

(1) For the daily variation (which means an "appeal to the cow" after 24 hours' interval)

$$U_d = \frac{M_p - M_w}{V_{d\,\max.}}.$$

(2) Similarly for the seasonal variation the "utility" is

$$U_s = \frac{M_p - M_w}{V_{s\,\max.}}.$$

When U is distinctly greater than unity, the method of detection is of some use, but if approximately equal to unity it is obviously useless for our purpose.

To illustrate this method, let us take the observations for refractive index $\Delta\mu$ recorded in Fig. 1 A. The maximum value of the daily variations is to be found in experiment Nos. 110 and 111, $\Delta\mu$ being 161 and 149 respectively (omitting the factor 10^4 which will cancel out in the ratio giving utility). The maximum $M_p - M_w$ is in experiment No. 121, M_p being 164 and M_w 141. Hence the highest measure of the utility of the method is

$$U_{d\,\max.} = \frac{M_p - M_w}{V_{d\,\max.}} = \frac{164 - 141}{161 - 149} = 1{\cdot}92,$$

which is just good enough to detect about three-fifths of the 10 per cent. added water (as will be explained later).

The lowest value of U is in experiment No. 119. It gives

$$U_{d\,\min.} = \frac{153 - 145}{12} = 0{\cdot}66,$$

so that in the latter case, although the milk sample contains 10 per cent. added water, it could not be detected by the refractive index method since U_d is well below unity. The method would, in fact, indicate that the farmer had withdrawn a considerable amount of the natural water of the milk.

In the same way it can be shown that relying on conductivity, or on rotation of plane of polarisation is equally unsafe, the daily variation of the quantities being comparable to the effect of 10 per cent. added water.

If, however, we examine in the same manner the curve given in Fig. 2, which expresses the quantity

$$M = \alpha\Delta\mu + \beta P + \gamma K$$

we notice a great improvement. It should first be stated that the value of these constants determined empirically were $\alpha = 10^4$, $\beta = 2675$ and $\gamma = 2 \times 10^4$.

For this method of testing, the maximum utility is

$$U_{d\,\text{max.}} = 6{\cdot}83,$$

and the minimum is $U_{d\,\text{min.}} = 3{\cdot}66.$

This method, therefore, never fails to detect added water by "appeal to the cow." In the worst cases it will detect three-quarters of the 10 per cent. added, whilst in the majority of cases it will discover seven-eighths of it, and in the easiest cases the whole of it. On the other hand, on trial it will be found that it is unsafe for the *seasonal* method of testing.

The "product" method (Fig. 3).

If we now turn to the product method ($\Delta\mu PK$) we notice from Fig. 3 that the seasonal changes, which are so marked in Fig. 1 A, B and C, and also to some extent in Fig. 2, are now absent; the main trend of the curve being parallel to the time axis. It becomes possible therefore to detect, and even to make a rough estimation of, added water at any time of the year from the "minimum value" of $\Delta\mu PK$ which in the curve is 18·1 in experiment No. 119. This, so far as the author knows, has not been possible hitherto.

Since the "maximum daily change" is 1·4 in Fig. 3 shown in Nos. 111 and 112, and the maximum seasonal change is 3·8, it is readily seen that the maximum and minimum values of the "utility" of this method are as follows:

$$U_{d\,\text{max.}} = 5{\cdot}3, \qquad U_{d\,\text{min.}} = 2{\cdot}85,$$
$$U_{s\,\text{max.}} = 2{\cdot}3, \qquad U_{s\,\text{min.}} = 1{\cdot}06.$$

This method, therefore, never fails even in the seasonal sense, as U_s is still above unity. As regards the "appeal to the cow" it also seems a satisfactory method, for in the worst cases it will detect two-thirds of the 10 per cent. added water and in the best cases the whole 10 per cent.

Estimation of the amount of added water by $\Delta\mu PK$ method.

As an example of a test by "appeal to the cow" according to this method let us consider the daily case Nos. 111 and 112. The suspected milk of No. 111 is to be tested by the pure milk of No. 112 obtained as a standard by the inspector the following day. Let $\Delta\mu PK = M_p$ be the measure of the standard, and let M_w be that for the suspected milk. As a protection for the farmer the maximum daily variation V_d must be assumed to have taken place during the 24 hours' interval. Then the amount of water W due with certainty to adulteration is given very simply by

$$W = \frac{1}{3}\frac{1}{M_p}(M_p - V_d - M_w).$$

Where $\frac{1}{3}$ is a factor introduced in view of the fact that the three quantities M_p, V_d and M_w are each a product of three factors $\Delta\mu$, P and K. Or, to express the added water in percentages,

$$\% \; W = \frac{100}{3M_p} (M_p - V_d - M_w).$$

So, we have for experiment Nos. 111 and 112, $M_p = 21$ for the standard, $M_w = 14{\cdot}2$ for the suspected milk and $V_d = 1{\cdot}4$. Therefore

$$\% \; W = \frac{100}{3 \times 21} (21 - 1{\cdot}4 - 14{\cdot}2) \doteqdot 8{\cdot}6\%$$

(whereas the actual adulteration was 10 per cent.).

THE METHOD OF RATIOS AND THE POSSIBILITY OF ITS APPLICATION TO HERDS OF ALL KINDS.

It is possible that the absolute magnitude of M may vary with the breed of the herd as already pointed out on p. 59. Now, if the ratio of M_1 and M_2 (namely the ratio of the values of M on successive days) be taken instead of their difference, the result should be independent of the breed. The maximum value of this ratio so far observed is 1·07. But to obtain a margin of safety it is taken as 1·08 throughout in this work. Thus if M_1/M_2 for the first and second days be unity, say (which means that the quality of the pure milk has not changed), then, the same quantity for the same herd measured on the second and third days and on the third and fourth days, etc., should not be greater than 1·08. And conversely, if the pure milks of the first and second days give $M_1/M_2 = 1{\cdot}08$, then the ratio for the pure milk of the second and third days cannot be less than unity, etc.

In both cases, it is the ratio of the greater to the lesser that is here considered. The arguments for this are as follows: in the first place, there is the same chance for the daily changes represented by M_1/M_2 to be equal to or *greater* than unity as to be equal to or *less* than unity. And in the second place, it is necessary, for the protection of the farmer, that the assumption should always be made that his milk of the previous day (which is regarded as being under suspicion) would have been giving naturally a pure M value of 1·08 *lower*, not higher, than his pure milk of to-day. There is, consequently, no need for both whole number and fractional values for this ratio. Either the one or the other will do, but to use both is inconvenient.

This ratio has been worked out from the figures obtained in Fig. 3. The result is shown in Fig. 4 A. In this curve it is found that the maximum value of the ratio for the daily determinations is 1·07 (*i.e.* in Nos. 111 and 112), but now regarded as 1·08. The ratio can exceed this figure if an interval of two or three days be allowed to elapse between the observations. Thus, there are

two instances, Nos. 130–131 and Nos. 133–134, in which the ratios are 1·16 and 1·13 respectively for three days' interval each. These two ratios are, however, so different from others of even longer intervals that there is some doubt as to their genuineness. But for the present, the curve must be taken as in the figure, as in any case we are only concerned with the 24-hour interval.

Hence, for the daily determinations on this herd, any whole milk, at any time of the year, that gives a ratio of M_1/M_2 greater than 1·08 must contain

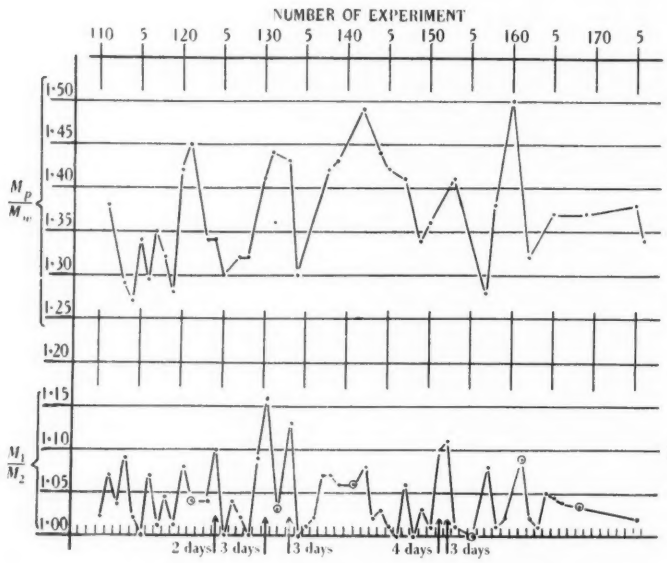


Fig. 4 B.

Fig. 4 A.

$\frac{M_1}{M_2}$ for pure milk and $\frac{M_p}{M_w}$ for 10 °/₀ watered milk.

added water. Moreover, since we are dealing with ratios, the statement is likely to be true of herds of all breeds and for all sizes of herds above eleven cows, though definite evidence on this point is not yet available.

The curve M_p/M_w (Fig. 4 B) shows the values obtained after adding 10 per cent. water to the milk used in the M_1/M_2 curve. There are considerable gaps in this curve due to the fact that the 10 per cent. water was not always added. Sufficient readings were taken, however, to exhibit the general effect of the added water.

The ratio method seems to give sufficient protection to the farmer and to the consumer, at least so far as the present series has revealed. The protection depends on the observed maximum of $M_p/M_w = 1{\cdot}5$ for 10 per cent. added water, and the observed maximum $M_1/M_2 = 1{\cdot}08$ for the daily natural variation of pure milk. The value of $M_p/M_w = 1{\cdot}5$ is seen in experiment No. 160 of Fig. 4 B. The effect on the milk of a given amount of water varies from day

to day. If the farmer is to be protected the maximum effect which may occur must be taken into account.

Different herds may lead to a slight modification of these actual figures, as they are based on observations on one herd only.

ESTIMATION OF THE ADDED WATER BY THE RATIO METHOD.

The easiest way of estimating the extent of adulteration is by the use of an ideal curve representing the effect of percentages of added water on the value of $\Delta\mu PK$. Such a curve is independent of the quality of the milk, and is shown in Fig. 5. The equation for it is approximately

$$y = 0{\cdot}0315x^{1{\cdot}2} + 1,$$

where x = the percentage added water, and $y = \Delta\mu PK$.

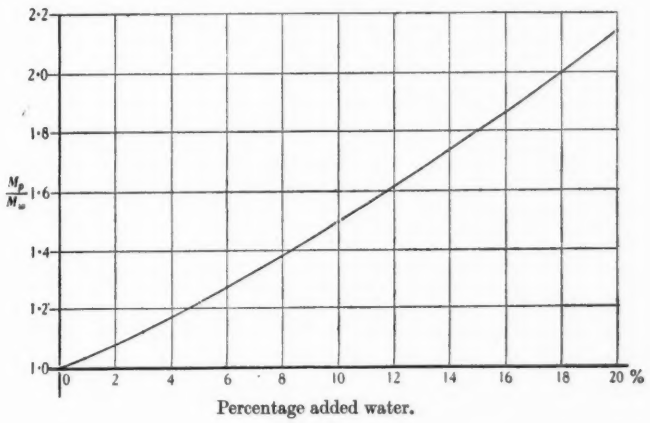


Fig. 5.

The curve has been constructed on a basis which gives the farmer requisite protection. Hence the maximum value of $M_p/M_w = 1{\cdot}5$ for 10 per cent. added water has been adopted.

The process of estimating the added water is as follows:

Let M_w be the value of $\Delta\mu PK$ for the suspected milk. Let M_p be the value of the same quantity of the pure milk obtained the following day. Then M_p/M_w represents the extent of adulteration.

Thus the adulteration of No. 111 may be tested by comparison with No. 112 (Fig. 3). Here $M_w = 14{\cdot}2$ and $M_p = 21$:

$$\frac{M_p}{M_w} = \frac{21}{14{\cdot}2} \div 1{\cdot}48.$$

On consulting Fig. 5, it is found that 1·48 corresponds to 9·6 per cent. added water.

The adulteration may also be calculated from the equation to the curve given above, which gives

$$\log x = 0{\cdot}833 \log \frac{y-1}{0{\cdot}0315},$$

where x = percentage added water, and $y = \frac{M_p}{M_w}$. Then

$$\log x = 0{\cdot}833 \log \frac{1{\cdot}48-1}{0{\cdot}0315} = 0{\cdot}833 \log 15{\cdot}2 = 0{\cdot}833 \times 1{\cdot}1818,$$

or $\log x = 0{\cdot}985$, and so $x \div 9{\cdot}66$ per cent. added water.

Similar comparisons may be made on Nos. 175 and 176. Here $M_w = 14$ and $M_p = 19{\cdot}7$. This gives the ratio $M_p/M_w = 19{\cdot}7/14 = 1{\cdot}41$; and this on Fig. 5 gives 8·45 per cent. added water.

The equivalent formula gives

$$\log x = 0{\cdot}833 \log \frac{1{\cdot}41-1}{0{\cdot}0315} \div 0{\cdot}833 \times 1{\cdot}139,$$

that is, $x \div 8{\cdot}87$ per cent. added water.

From the above examples (the one representing observations made in January and the other in August) it will be observed that the ratio method gives sufficiently reliable results, considering the seasonal variations in the characteristics of the milk exhibited by $\Delta\mu$, P and K in Fig. 1.

Tests independent of "appeal to the cow."

The method of testing for added water described above involves an "appeal to the cow." It is of interest to examine whether such an "appeal" can be safely dispensed with. The few trials that have already been made to determine whether the level of the curve of Fig. 3 is likely to be much affected by varying breeds have shown a negative result. If the results of these preliminary trials are ultimately substantiated, we can assume without serious error that the value $\Delta\mu PK = 19$ (Fig. 3) may be considered as a datum line for the whole season. This value gives ample protection to the farmer, since the difference between 19 and the minimum value 18·1 observed in No. 119 is less than the maximum daily variation of $\Delta\mu PK$, which is 1·4.

If, therefore, M_w is the $\Delta\mu PK$ of the milk under suspicion, $19/M_w$ represents the adulteration that can be measured by this method, using Fig. 5 for the purpose.

As an example, consider the case of No. 113, which is considered anomalous and the most difficult in the series. Here $M_w = 16{\cdot}9$, and $19/M_w = 1{\cdot}13$. From Fig. 5 it will be seen that this value represents about 3·2 per cent. of added water instead of the 10 per cent. actually present. On the other hand, No. 142 (Fig. 3) gives $M_w = 12{\cdot}6$, and $19/M_w = 1{\cdot}5$. This corresponds on Fig. 5 to 10 per cent. added water.

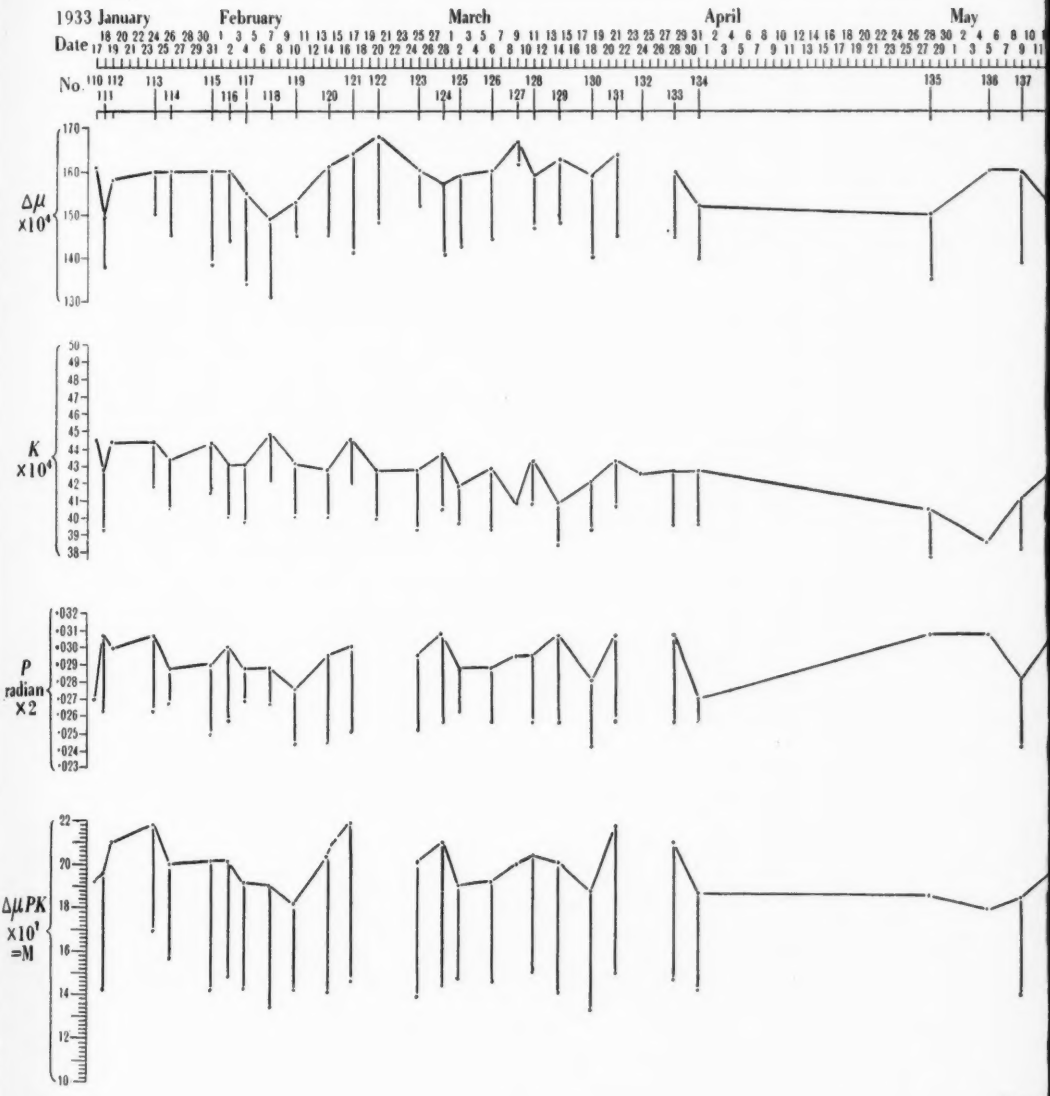


Fig. 6

Here observations are shewn with abscissae proportional to tim

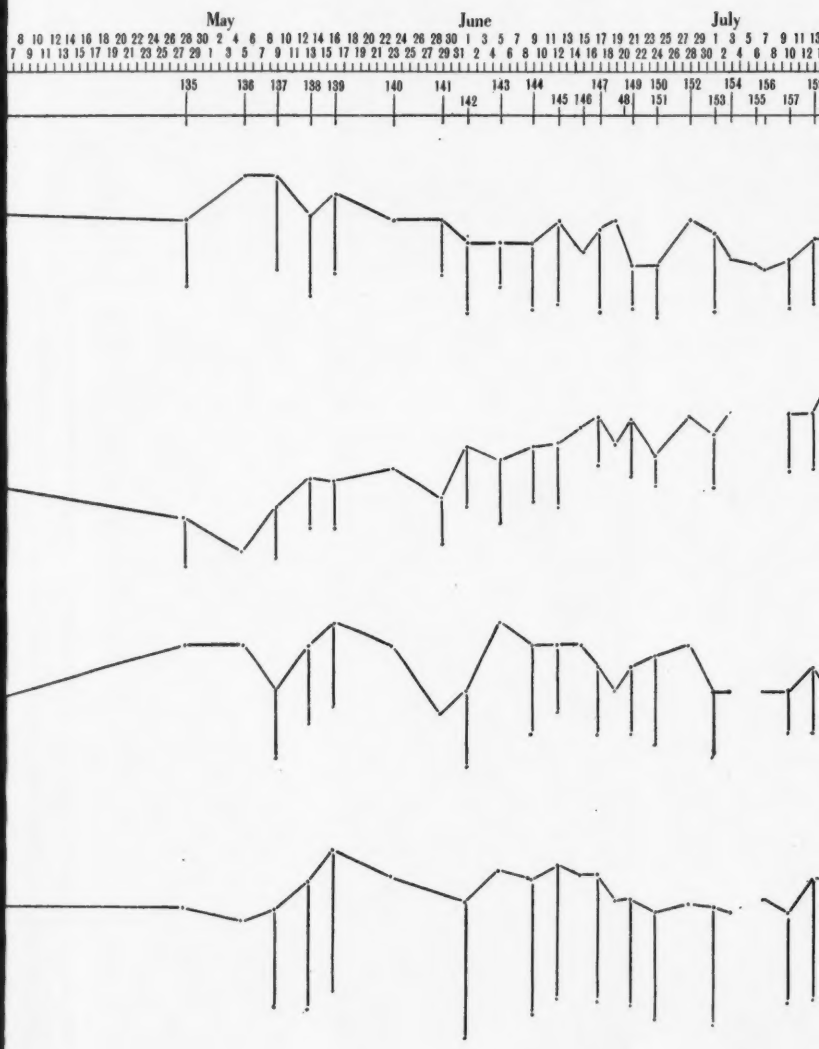
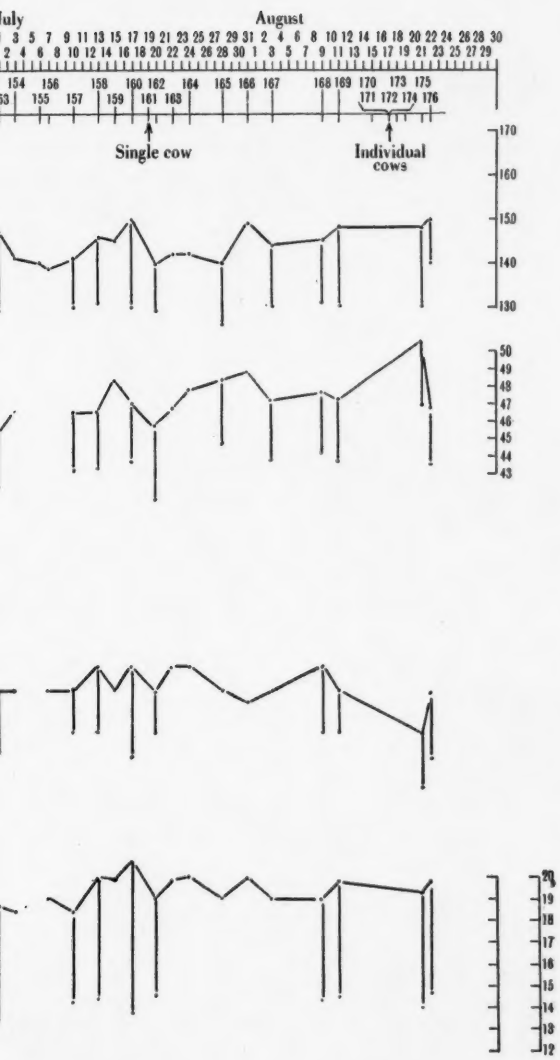


Fig. 6.

wn with abscissae proportional to time. In other respects curves are same as in Figs. 1 and 3.



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and 3.

The utilities U_d and U_s for this ratio method are satisfactory, but cannot be expressed in the same way as in the previous methods. Instead of $\frac{M_p - M_w}{V}$ we must write $\frac{M_p/M_w}{V}$, the V now becoming M_1/M_2. Similarly for the seasonal cases, V becomes

$$\frac{M_3}{M_4} = \frac{\text{Max. Seasonal } M}{\text{Min. Seasonal } M},$$

so, for the daily cases

$$U_d = \frac{M_p/M_w}{M_1/M_2} = \frac{M_p/M_w}{1{\cdot}08},$$

and for the seasonal

$$U_s = \frac{M_p/M_w}{M_3/M_4} = \frac{M_p/M_w}{1{\cdot}21}.$$

On referring to Fig. 3 it will be seen that for this ratio method, U expressed as above gives

$$U_{d\,\text{max.}} = 1{\cdot}38, \qquad U_{d\,\text{min.}} = 1{\cdot}185,$$

$$U_{s\,\text{max.}} = 1{\cdot}24, \qquad U_{s\,\text{min.}} = 1{\cdot}06.$$

These figures are not comparable with those given by $\frac{M_p - M_w}{V}$; nevertheless, the sensitiveness of the ratio method is much the same as the $\Delta\mu PK$ method and is dependent on it. Hence for convenience of comparison, the values above for U may be multiplied by 5.

The important point here is the fact that no great difference now exists between $U_{d\,\text{min.}}$ and $U_{s\,\text{min.}}$ which implies that we may obtain a fairly good measure for adulteration without "appeal to the cow."

SUMMARY.

The various methods tested in this work have revealed the criteria of utility, U, shown in the following table for the detection of added water as measured by $\frac{M_p - M_w}{V_d}$ and $\frac{M_p - M_w}{V_s}$ for the "difference" modes, and for the ratio modes by $\frac{M_p/M_w}{M_1/M_2}$ and $\frac{M_p/M_w}{M_3/M_4}$, where M_3/M_4 is the ratio of the maximum and minimum seasonal values of M. All the figures are for 10 per cent. added water. Utility values equal to and less than unity in the table are useless.

Comparison of "utilities."

Method	Daily (U_d)		Seasonal (U_s)	
	Max. $\frac{M_p - M_w}{V_d}$	Min. $\frac{M_p - M_w}{V_d}$	Max. $\frac{M_p - M_w}{V_s}$	Min. $\frac{M_p - M_w}{V_s}$
$\Delta\mu$	2·0	0·8	0·82	0·21
P	1·34	0·6	0·83	0·37
K	2·86	0·96	0·5	0·25
$\alpha\Delta\mu + \beta P + \gamma K$	6·83	3·66	1·46	0·78
$\Delta\mu PK$	5·3	2·85	2·3	1·06
$(\Delta\mu PK)_m/(\Delta\mu PK)_w$	1·38	1·185	1·24	1·06
Same multiplied by 5	6·9	5·92	6·2	5·3

It will be observed in the table that $\Delta\mu$, P and K used independently are uncertain as methods of detection, for they are safe only in the first column. The second column shows that they may be unsafe at any time of the year even with "appeal to the cow." For seasonal determinations they are all useless.

The $\alpha\Delta\mu+\beta P+\gamma K$ method is quite safe for "appeal to the cow" at any time of the year, but fails in the last column, showing that it is uncertain for seasonal determinations independent of the daily "appeal." It exhibits a greatly increased utility compared with the $\Delta\mu$, P and K methods used separately.

The $\Delta\mu PK$ also gives a high utility and for the first time we see the four columns with utilities all above unity. For the first two columns, that is, for "appeal to the cow," the figures are such that it is perfectly safe, though not quite as sensitive as the previous method. But, on the other hand, we have now a method that is just beginning to be useful in the seasonal sense, for the last column is above unity.

The ratio method $\frac{(\Delta\mu PK)_m}{(\Delta\mu PK)_w}$ is still better, for now we have values of utility well above unity throughout, with no great difference between the seasonal and the daily utilities. In this case the utility has got to be expressed as the ratios of the quantities throughout as already stated above, and are therefore not comparable with the figures for the five previous methods. The actual sensibility of the method is much the same as for the product $\Delta\mu PK$ method, for it depends on that product. By multiplying throughout by 5 the figures become comparable with the rest in the table. These are given in the last line of the table. It will now be seen that no great difference exists between the last column in the "seasonal" and the second column in the "daily." This means that we may obtain a fairly good measure for adulteration without "appeal to the cow."

From the study of the curves we may infer:

(1) That $\Delta\mu$, P and K are probably measures of three different and perhaps independent characteristics of milk.

(2) That the product $\Delta\mu.K$ tends towards a constant, being in cases of substantial daily changes distinctly in opposite phase relations.

(3) That $P.K$ tends towards a constant.

(4) That the effect of added water is to put all three in the same phase.

From this it may be concluded that the added water does not act in the same manner as the natural liquid of the milk. Changes in the characteristics of the milk's own liquid are largely compensated for in the product $\Delta\mu.P.K$ with a decided tendency to a constant, whereas the added water is never compensated for. This fact could never be discovered by attending to a single characteristic, whether it be fat content, non-fatty solid content, refractive index, conductivity or freezing-point.

There are, however, three cases, in the whole series dealt with in this work,

in which all three, $\Delta\mu$, P and K, are in the same phase, and yet the product in each case comes within the same range of values as the rest, and so,

(5) We conclude that the absolute values of the products also tend to a constancy, at least for this particular herd.

In fact, the three characteristics of the milk may be said to vary in concentration relatively among themselves, in such a way that as one increases, another generally decreases, with a tendency to a steady condition as Figs. 2 and 3 prove to be the case. But an alteration of concentration by the addition of water cannot but affect all three characteristics in the same manner. Consequently there is a real discrimination possible between the natural variation in the characteristics of the milk and that brought about artificially by the added water. That is true for the three types of detecting methods here considered, viz. $\alpha\Delta\mu+\beta P+\gamma K$, $\Delta\mu PK$ and $\frac{(\Delta\mu PK)_m}{(\Delta\mu PK)_w}$. On the other hand, the usual methods of detection are all methods of measuring dilution only, unattended by any discrimination between the added water and the natural changes in the milk's constitution.

The milk used in this work was obtained from Miss M. A. Morris, Piercefield Dairy Farm. It was a part of that usually delivered for household use. No special care was taken beyond that observed in the daily routine of the dairy duties.

ACKNOWLEDGMENT.

These experiments were carried out in the laboratories of the Physics Department of the University College of Wales, Aberystwyth. The author must record here his gratitude to Prof. Gwilym Owen for his ever ready advice, his lively encouragement, and help with the text. Also to Prof. Fagan and Prof. Jones Griffith of the Agricultural Laboratories and to Dr Ernest Jones, M.O.H. for Cardiganshire, for valuable discussions during the course of the experiments.

A word of caution is needed here in respect to the measure of P. Its unit has not yet been decided on. The angle of rotation is given throughout in fractions of a radian as measured from a polarimeter cell of only 4 cm. in length. It is hoped that this may be considerably lengthened under improved conditions in future measurements. The present levels of the curves in which P is involved may, therefore, not be final. That, however, will not affect the "utility" values of the methods here described.

APPENDIX.

Data for herd of 11 *cows.*

Date	No.	μ Pure	μ Watered	$K \times 10^4$ Pure	$K \times 10^4$ Watered	P (radian) Pure	P (radian) Watered	Remarks
1932								
Oct. 6	74	1·3481	1·3462	45·87	42·55	0·027	—	
7	75	1·3479	1·3466	46·51	43·1	0·0295	0·0256	
18	76	1·3490	1·3474	44·25	41·3	0·0295	0·0256	
22	77	1·3480	1·3464	45·5	41·67	0·0280	0·023	
24	78	1·3480	1·3469	44·95	41·67	0·0281	0·0255	
25	79	1·3482	1·3469	45·25	41·84	0·0290	0·0212	P_p here doubtful. Value estimated from P_w
26	80	1·3489	1·3470	44·05	40·98	—	—	Serum turbid
27	81	1·3491	1·3480	44·44	41·32	0·0270	0·0192	
29	82	1·3494	1·3485	44·6	41·67	0·0385	0·0334	
31	83	1·3497	1·3486	43·5	40·32	0·0256	0·0218	
Nov. 2	84	1·3495	1·3480	44·0	40·98	0·0282	0·0243	
4	85	1·3490	1·3476	43·67	40·98	0·0275	0·0250	
5	86	1·3492	1·3480	43·67	40·82	0·0295	0·0250	
7	87	1·3493	1·3475	45·05	42·1	0·027	0·023	
9	88	1·3494	1·3480	45·25	42·02	0·027	0·0225	
10	89	—	—	—	—	—	—	Single Jersey cow
24	90	1·3490	1·3476	44·2	41·32	—	—	P could not be obtained, serum too turbid
26	91	1·3490	1·3475	46·51	43·48	0·03075	0·0275	
28	92	1·3490	1·3480	42·55	39·29	0·0334	0·0275	
30	93	1·3493	1·3480	43·67	40·98	0·0334	0·0275	
Dec. 1	94	1·3496	1·3474	41·67	39·22	0·0320	0·0275	
3	95	1·3496	1·3477	41·67	39·06	0·0289	0·026	
5	96	1·3491	1·3472	42·74	40·0	0·0307	0·0256	
7	97	1·3490	1·3479	44·44	41·49	0·0307	0·0263	
9	98	1·3492	1·3479	43·67	40·98	0·0340	0·03075	
12	99	1·3496	1·3475	42·55	39·22	0·0275	0·0244	
14	100	1·3491	1·3477	43·29	40·0	0·03075	0·0244	
20	101	—	—	—	—	—	—	An exp. carried out at 14·3° C. instead of 20·0° C. Not to be included
21	102	1·3493	1·3483	43·48	41·67	0·0281	0·0237	
24	103	1·3494	—	43·50	—	—	—	An exp. in which μ and K only were required
1933								
Jan. 7	104	1·3487	—	43·5	—	—	—	Exps. on effect of time on μ and K readings
9	105	1·3490	—	44·0	—	—	—	
10	106	1·3485	1·3470	43·2	41·7	0·0281	0·0237	
11	107	1·3490	1·3473	45·1	42·0	0·0282	0·0244	
12	108	1·3488	1·3470	43·5	41·0	0·0275	0·0205	
13	109	1·3483	1·3473	44·20	41·0	0·0281	0·0244	
17	110	1·3491	—	44·50	—	0·0269	—	
18	111	1·3479	1·3468	42·74	39·37	0·03075	0·02625	
19	112	1·3488	—	44·44	—	0·030	—	
24	113	1·3490	1·3480	44·44	41·67	0·0307	0·0270	
26	114	1·3490	1·3488	43·48	40·50	0·0288	0·02685	
31	115	1·3490	1·3468	44·44	41·49	0·0290	0·0250	
Feb. 2	116	1·3490	1·3474	43·1	40·0	0·030	0·0256	
4	117	1·3485	1·3464	43·1	39·68	0·0288	0·0269	
7	118	1·3479	1·3461	44·84	41·84	0·0288	0·0266	
10	119	1·3483	1·34675	43·1	40·0	0·0275	0·0243	
14	120	1·3491	1·3475	42·74	40·0	0·0295	0·02435	
17	121	1·3494	1·3471	44·64	41·67	0·030	0·0250	
20	122	1·3498	1·3478	42·74	40·0	—	—	
25	123	1·3490	1·3410	42·74	39·37	0·0295	0·0250	
28	124	1·3487	1·3470	43·67	40·49	0·03075	0·0256	
Mar. 2	125	1·3489	1·3410	41·84	39·68	0·0288	0·0262	
6	126	1·3490	1·3474	42·92	39·37	0·0281	0·0256	
9	127	1·3497	—	40·82	—	0·0295	—	

Data for herd of 11 *cows* (continued).

Date 1933	No.	μ Pure	μ Watered	$K \times 10^4$ Pure	$K \times 10^4$ Watered	P (radian) Pure	P (radian) Watered	Remarks
Mar. 11	128	1·3489	1·3477	43·48	40·82	0·0295	0·0256	
14	129	1·3493	1·3478	40·98	38·46	0·03075	0·0256	
18	130	1·3489	1·3470	42·1	39·37	0·0281	0·0243	
21	131	1·3494	1·3475	43·48	40·65	0·03075	0·0256	
24	132	—	—	42·55	—	—	—	Exp. cut short
28	133	1·3490	1·3475	42·74	39·68	0·03075	0·0256	
31	134	1·3482	1·3470	42·74	39·68	0·0269	0·0256	?
Apr. 28	135	1·3480	1·3465	40·5	37·88	0·03075	—	
May 5	136	1·3490	—	38·61	—	0·03075	—	
9	137	1·3490	1·3469	41·32	38·46	0·0281	0·0243	P_w here estimated from P_p
13	138	1·3481	1·3463	42·92	40·0	0·03075	0·0262	" "
16	139	1·3486	1·3468	42·74	40·0	0·0320	0·0269	" "
23	140	1·3480	—	43·48	40·5	0·03075	—	
29	141	1·3480	1·3468	41·67	39·0	—	—	P could not be read due to turbidity
June 1	142	1·3475	1·3459	44·60	41·15	0·0281	0·0238	P_w estimated from P_p
5	143	1·3475	1·3465	43·86	40·98	0·0320	0·0272	" "
9	144	1·3475	1·3460	44·64	41·32	0·03075	0·0256	
12	145	1·3480	1·3461	44·84	40·98	0·03075	0·0269	
15	146	1·3473	—	45·87	—	0·03075	—	
17	147	1·3478	1·3459	46·30	43·48	0·0295	0·0256	
19	148	1·3480	—	44·84	—	0·0281	—	
21	149	1·3470	1·3470	46·08	42·74	0·0295	0·0256	
24	150	1·3470	1·3458	43·95	42·37	0·0301	0·0250	
24	151	1·3470	—	45·66	—	0·0295	—	
28	152	1·3480	—	46·3	—	0·03075	—	
July 1	153	1·3477	1·3459	45·25	42·19	0·0281	0·02435	
3	154	1·3471	—	46·51	—	0·0281	—	
6	155	1·3470	—	47·8	—	—	—	
7	156	1·3469	—	49·0	—	0·0281	—	
10	157	1·3471	1·3460	46·51	43·1	0·0281	0·0256	
13	158	1·3476	1·3461	46·5	43·3	0·0295	0·0256	
15	159	1·3475	—	48·3	—	0·0281	—	
17	160	1·3480	1·3460	47·06	43·67	0·0295	0·0243	
19	161	—	—	—	—	—	—	Single Jersey cow
20	162	1·3470	1·3459	45·33	41·49	0·030	0·0256	
22	163	1·3472	—	46·73	—	0·0295	—	
24	164	1·3472	—	47·85	—	0·0295	—	
28	165	1·3470	1·3456	48·3	44·64	0·0281	0·0237	
31	166	1·3479	—	—	—	0·0275	—	Balance by earphone difficult due to storm
Aug. 3	167	1·374	1·3460	47·17	43·86	0·0281	0·0182	
9	168	1·3475	—	47·62	—	0·0295	—	
11	169	1·3478	1·3460	47·17	43·67	0·0281	0·0256	
15	170	—	—	—	—	—	—	Single Jersey cow
15	171	—	—	—	—	—	—	" "
17	172	—	—	—	—	—	—	" "
18	173	—	—	—	—	—	—	" "
19	174	—	—	—	—	—	—	" "
21	175	1·3478	1·3460	50·5	46·95	0·0256	0·0230	
22	176	1·3480	1·3470	46·73	43·48	0·0281	0·0243	

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94. A STUDY OF THE TITRATABLE ACIDITY OF MILK

I. THE INFLUENCE OF THE VARIOUS MILK BUFFERS ON THE TITRATION CURVES OF FRESH AND SOUR MILK

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(With 10 Figures.)

Introduction.

The titratable acidity of fresh milk has been studied by several investigators, but the effects of the various buffers present have not been precisely defined. Early statements as to the cause of the titratable acidity were expressions of opinion unsupported by experimental evidence; the theories of indicators and buffer action were not considered. The acids and bases present were calculated by arbitrary rules to various combinations of salts, and the acidity was stated to be due to the presence of acid salts in these hypothetical mixtures.

Van Slyke and Bosworth (1) recognised the importance of the calcium of the milk in the titration. They claimed that the acidity was due only to the phosphates and that these titrate inaccurately in the presence of calcium owing to the precipitation of tribasic calcium phosphate as the solution is made alkaline, whereas in the absence of calcium the dibasic phosphates are neutral to phenolphthalein and their formation marks the end of the titration. These authors considered that the casein in the milk was combined with a definite proportion of calcium and this was unaffected during the titration. Later work has shown how the base bound by the protein increases with *p*H.

Rice (2), McInerney (3), Rice and Markley (4), and others have shown that fresh milks of high titratable acidity are high in the proportion of total solids and ash present. Rice and Markley, from the analyses of a number of milks, come to the conclusion that the casein contributes 0·05–0·08 per cent. acidity to the milk or about half the total titration. The citrate, carbonate and lactalbumin each contribute about 0·01 per cent. and the remainder is due to phosphate. They do not consider the possible interactions of the various ions, and it will be shown that a simple addition of the buffers does not account for the effects observed.

Harris (5) states that if the titration curves of the main buffers (casein and lactalbumin, phosphates and citrates) are plotted, making the abscissae proportional to the percentage present, the curve obtained by the summation of these separate curves agrees excellently with that for milk. He gives no experimental results to support this statement and the author could not confirm it.

Preliminary work showed that the reactions in the titration are not so simple as has often been assumed, and it was accordingly decided to investigate the subject from a fresh angle. This was to study first the titration of simple calcium-phosphate solutions under conditions similar to the milk titration, then to extend the work by the addition of citrate to these solutions, and finally to study the titration when casein as well as phosphate, citrate and calcium was present. The carbonate, lactalbumin and magnesium have not been considered in the experimental work. They are present in comparatively small proportions, and it was found that the work on the major constituents explained all the points of the titration.

The titrations were made between about *p*H 4·8 and 8·0. The former figure corresponds approximately to a sour milk and the latter to the phenolphthalein end-point. In titrating milk the end-point actually occurs at about *p*H 8·5, but above *p*H 8·0 the buffering is slight and conclusions based on an end-point of *p*H 8·0 may be safely applied to the phenolphthalein titration. For purposes of discussion *p*H 6·6 will be taken as typical of fresh milk.

EXPERIMENTAL METHODS.

Ten cubic centimetre portions of each of the solutions being titrated were put into test-tubes, measured quantities of sodium hydroxide solution (0·4*N*) added and the solutions thoroughly mixed. As the object of the experiments was to investigate the reactions occurring during the ordinary titration of milk no attempt was made to reach a final state of equilibrium. It was found, however, that if *p*H measurements were taken immediately after the addition of sodium hydroxide extremely erratic and unreproducible readings were obtained, whereas if the solutions were allowed to stand 15 min. the results could be duplicated. Unless otherwise stated, therefore, the *p*H readings recorded were obtained 15 min. after the addition of sodium hydroxide. Admittedly this is not an exact imitation of the usual rapid titration, but it approximates to it.

*p*H was measured by the quinhydrone method. The temperature was $20 \pm 2°$ C., no attempt being made to work within closer limits. Precautions were taken to have the solutions carbonate free.

THE TITRATION OF SOLUTIONS CONTAINING PHOSPHATES AND CALCIUM.

The titration curve of phosphoric acid with solutions of calcium hydroxide has been studied by Wendt and Clark (6), Hoffmann and Gortner (7), Holt, La Mer and Chown (8), Farnell (9) and Britton (10). These investigations have established that in the titration of phosphoric acid with lime water precipitation occurs soon after one equivalent of base is present, at a *p*H which depends on the temperature, ionic strength of the solution and time of standing, but which is of the order of 5–6·8. The immediate precipitate is $CaHPO_4$, which on long standing at a *p*H as low as 5, or more rapidly at high *p*H, is converted

to $Ca_3(PO_4)_2$ or even more basic salts. This precipitation of the phosphate prevents a rapid rise in *p*H, and even after three equivalents of $Ca(OH)_2$ are added the *p*H is 8–9. This is the point at which the phenolphthalein end-point is obtained in titrations, so that the phosphoric acid titrates as a tribasic acid to this indicator with calcium hydroxide. With sodium hydroxide it titrates as a dibasic acid, a sharp end-point being obtained when sufficient base has been added to form Na_2HPO_4.

The titrations described above are not, however, quite analogous to that of milk, where a solution containing phosphates and calcium in solution is titrated with sodium hydroxide. The behaviour of such a solution was investigated in the present work, the ratio of phosphoric acid to calcium being varied but the concentrations being of the same order as those occurring in milk.

A stock solution was prepared, using boiled distilled water, of 16·10 g. of A.R. dihydrogen potassium phosphate per litre. The theoretical content of P_2O_5 in this solution is 0·839 g. per 100 ml., and an analysis showed 0·841 g. per 100 ml.

A stock calcium solution was prepared by adding a slight excess of Kahlbaum's pure precipitated calcium carbonate to 179 ml. of 2*N* HCl, boiling, filtering and diluting to 1 litre. After analysis this was diluted so as to contain calcium equivalent to 1 g. of CaO per 100 ml. and the concentration checked by analysis.

The phosphate-calcium solutions for titration were prepared by mixing the required quantities of phosphate and calcium stock solutions and making to 100 ml. with boiled distilled water.

Fig. 1 shows the results of titrations performed in the manner described on solutions of the composition given in Table I.

Table I.

No.	P_2O_5 per 100 ml.	CaO per 100 ml.	Ratio CaO/P_2O_5
1	0·168	Nil	0
2	0·168	0·066	0·394
3	0·168	0·099	0·590
4	0·168	0·1325	0·789
5	0·168	0·165	0·984
6	0·168	0·198	1·18

It should be noted that the ratio CaO/P_2O_5 for the monobasic salt $Ca(H_2PO_4)_2$ is 0·394, for $CaHPO_4$ 0·789, and for $Ca_3(PO_4)_2$ 1·18.

In Fig. 1 the sodium hydroxide used in the titration is converted to and recorded as ml. *N*/1 NaOH per 100 ml. of solution.

Curve 1 is that portion of the well-known titration curve of phosphoric acid lying between the neutralisation of the first and second hydrogen atoms. The maximum buffering occurs at about *p*H 6·9, when NaH_2PO_4 and Na_2HPO_4 are present in equal concentrations. The dissociation constant of phosphoric acid which is functioning here corresponds to the reaction $H_2PO_4' \rightleftharpoons H^{\cdot} + HPO_4''$, and is $pK_2 = 6{\cdot}9$.

The effects of increasing concentrations of calcium on the titration are

shown by curves 2–6. The *p*H at which maximum buffering occurs decreases with increase of calcium concentration. This is due to the greater calcium-ion concentration which favours precipitation of the phosphate at the lower *p*H. As the other work in this field has shown, this precipitation of the phosphate caused a flattening in the titration curve. When a longer period of time elapsed between the addition of the sodium hydroxide and the measurement of *p*H this flattening was more pronounced, and more sodium hydroxide was required to bring the solution to *p*H 8. As an example the curve 7 in dotted line shows the titration of a similar mixture to curve 5 when 24 hours were allowed to elapse between the addition of the sodium hydroxide and measurement of *p*H. The dotted line cutting curves 2–6 shows the approximate point at which precipitation of calcium phosphate commenced.

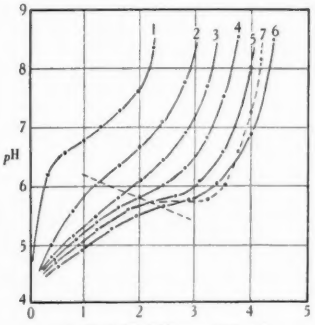

Fig. 1.

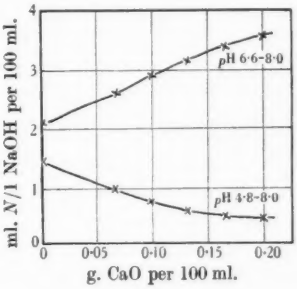

Fig. 2.

The quantities of alkali needed to bring the solutions from *p*H 6·6 to 8·0 and from *p*H 4·8 to 8·0 are shown in Fig. 2. The lower curve corresponds to the titration of fresh milk and the upper one to sour milk if fresh milk is arbitrarily considered to have a *p*H of 6·6 and sour milk a *p*H of 4·8. It will be noted that with these comparatively simple phosphate-calcium solutions the base required to shift the *p*H from 6·6 to 8·0 varies inversely with the calcium concentration, whereas when the shift from *p*H 4·8 to 8·0 is considered it varies directly with the calcium concentration. As has been pointed out the increase in the latter case is due to the precipitation of the more basic phosphate. The curves show that the decrease of base with increase of calcium from *p*H 6·6 to 8·0 is due to the precipitation of the phosphate which occurs at lower *p*H, the flatter portion of the curves being below *p*H 6·6. By the time this is reached the curves have become increasingly steep with increase of calcium concentration.

The above results give a general indication of the effect of adding NaOH to simple phosphate-calcium systems. The work with more complex solutions, which approach the composition of milk, will show how greatly their properties are modified by the other constituents.

The titration of solutions containing citrates in addition to phosphates and calcium.

The next stage in the scheme for studying solutions which step by step approach the composition of milk was to add citrates to the system.

A stock solution of A.R. trisodium citrate ($2Na_3C_6H_5O_7$, $11H_2O$) containing 18·59 g. per litre, equivalent to 1 g. of citric acid per 100 c.c., was prepared with recently boiled distilled water. This, together with the calcium and phosphate solutions described in the previous section, was used for preparing the solutions to be titrated. The titrations were performed in exactly the same manner as for the phosphate-calcium mixtures.

Fig. 3 shows the titration of solutions of the composition given in Table II.

Table II.

No.	P_2O_5 per 100 ml.	CaO per 100 ml.	Citric acid per 100 ml.
1	0·168	0	0
2	0·168	0·165	0
3	0·168	0·165	0·100
4	0·168	0·165	0·200
5	0·168	0·165	0·300

It will be noted that the concentrations of phosphate and calcium have been kept constant at 0·168 and 0·165 g. per 100 ml. respectively, figures typical of milk, and the concentration of citrate has been varied. Curves 1 and 2 are included for comparison.

Before discussing these curves it will be well to consider briefly the titration of citric acid and the dissociation of calcium citrate.

Hastings and Van Slyke (11) give a typical titration of citric acid with sodium hydroxide and deduce the dissociation constants to be $pK_1=3·08$, $pK_2=4·39$, $pK_3=5·49$. The three dissociation constants are so close that no well-defined breaks occur in the titration curve as the three hydrogen atoms are neutralised. The *p*H increases regularly with increase of sodium hydroxide until 2·8 equivalents of base are added, when the *p*H is 6·0. An additional 0·1 equivalent of base brings the *p*H to 6·5, when it rises sharply to above *p*H 8·0 as the full three equivalents are added. The buffer effect of a citric acid-sodium citrate solution is thus fairly constant between *p*H 2·5 and 6·0, and then rapidly decreases to almost nil above *p*H 6·5.

The properties of a solution containing calcium are known to be affected by the addition of citrates, and it has often been stated that this is due to the production of unionised calcium citrate. The author (Wiley (12)) found that at a concentration corresponding approximately to that of citric acid in milk (0·2 g. per 100 ml.) calcium citrate was about 15 per cent. dissociated in solutions free from other salts. The degree of dissociation (0·15) is thus quite low for a salt of calcium, but calcium citrate is by no means "undissociated." However, a simple experiment described by Whittier (13) shows that this low

dissociation is of great significance in the salt equilibrium in milk. A solution containing 0·0465 g. of calcium per 100 ml. and 0·142 g. of P_2O_5 per 100 ml. precipitated calcium phosphate at *p*H 6·6. When 0·237 g. of citric acid per 100 ml. was present, however, precipitation did not occur.

The curves of Figs. 3 and 4 show how the citrate has modified the phosphate-calcium titration. The *p*H at which maximum buffering occurs increases with increasing concentration of citrate. The buffering is due to the precipitation of calcium phosphate, and the citrate prevents this until lower hydrogen-ion concentrations are reached. This effect is undoubtedly due partly to the increased ionic strength of the solutions with the trivalent citrate ions and partly to the diminished calcium-ion concentration because of the low dissociation of calcium citrate. The result is that the titration from *p*H 6·6 to 8·0 shows an increase of sodium hydroxide with increase of citrate present. When the citrate concentration increases from 0·1 to 0·3 per cent., the sodium hydroxide necessary to titrate between these limits is doubled.

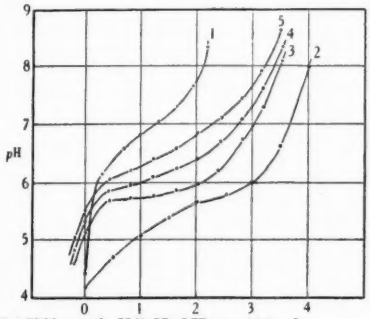

Fig. 3.

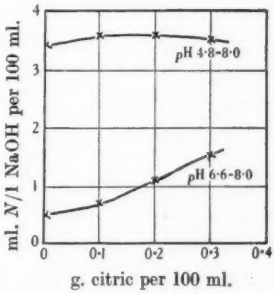

Fig. 4.

This is an excellent example of the impossibility of predicting the titratable acidity of milk by the simple addition of the titrations of its components. Citrates have no buffering effect at *p*H 6·6 and higher, but, as is shown here, when citrates are added to phosphate-calcium mixtures, the resulting titration curve is greatly modified.

The titration from *p*H 4·8 to 8·0 shows another interesting point. The total quantity of sodium hydroxide needed is very little influenced by the proportion of citrates used in these experiments. The effect on the retardation of phosphate precipitation should not change the gross amount of alkali needed, and at first glance one might expect the additional buffer action of the citrate between *p*H 4·8 and 6·5 to add to the other buffer effects and give an increased titration with increase of citrate. At *p*H 4·8 citric acid when titrated with sodium hydroxide is approximately two-thirds neutralised, and another equivalent of base is needed to bring it to the tribasic salt.

The low dissociation of calcium citrate also affects this titration, as can

be seen from the following considerations. The usual equation describing the course of titration of a weak acid with a strong base is

$$p\mathrm{H} = pKa + \log \frac{\text{salt}}{\text{acid}}. \qquad \text{.....(1)}$$

This follows immediately from the equation

$$p\mathrm{H} = pKa + \log \frac{(A')}{(HA)}, \qquad \text{.....(2)}$$

on the assumption of a slightly dissociated acid and completely dissociated salt ((A') = concentration of anion and (HA) = concentration of undissociated acid).

When equation (1) is applicable, if the acid is 99 per cent. neutralised, $p\mathrm{H} = pKa + 2$, *i.e.* the pH near exact equivalence is 2 units higher than the pK of the acid.

If a salt of the acid can be formed which is but slightly dissociated the ratio $(A')/(HA)$ must be smaller, and consequently the pH at which the acid is nearly neutralised by base must be lower. In the case of the calcium-citric acid reaction here considered the buffering of the acid has been shifted to the acid side of pH 4·8, and consequently the citrate buffering above this figure is negligible.

Perhaps a simpler way of arriving at the same conclusion is to consider the equation

$$2H_3C_6H_5O_7 + 3CaCl_2 \rightleftharpoons Ca_3(C_6H_5O_7)_2 + 6HCl.$$

Calcium citrate being but slightly dissociated, the equation will tend to proceed to the right. The titration curve of a solution of citric acid containing calcium chloride will therefore tend to approach that of the strong acid (HCl), and buffering will be slight in the region under consideration, pH 4·8–8·0. This conclusion was demonstrated by the following simple experiment. A solution of citric acid was titrated with sodium hydroxide using methyl red as indicator. This changes colour at about pH 5·0. A vague end-point was attained when the acid was about two-thirds neutralised. A solution of calcium chloride was added to a citric acid solution of similar concentration before titration, and a sharp end-point was obtained at complete neutralisation with the same indicator. Both the critic acid and citric acid *plus* calcium chloride, when titrated to the phenolphthalein end-point, required the same quantity of sodium hydroxide.

Hence the experimental titration curves show that the addition of citrates to phosphate-calcium solutions modify the titration between the limits of pH 6·6 and 8·0 and pH 4·8 and 8·0 in a manner quite opposite to that deduced by a simple addition of the titration curves of citrate and phosphate-calcium solutions separately. In both cases the results obtained are opposite to those to be expected by this addition.

These titrations were further investigated by making up a series of solutions in which the concentrations of phosphate and citrate were kept constant and

the concentration of calcium was varied. The solutions were prepared and titrations performed in the manner already described.

The results are shown in Figs. 5 and 6. Table III gives the concentrations of the solutions.

These curves confirm in a general manner the results already described. As the calcium concentration increases, the *p*H at which maximum buffering occurs decreases, but, owing to the presence of the citrate, this is higher than in corresponding solutions in which citrate is absent. In curve 3 the calcium is present in approximate equivalence to the citrate, but in curves 4, 5 and 6 it is present in excess and the *p*H of maximum buffering is correspondingly

Table III.

Curve	P_2O_5	Citric acid	CaO g. per 100 ml.
1	0·168	0	0
2	0·168	0·2	0
3	0·168	0·2	0·0997
4	0·168	0·2	0·133
5	0·168	0·2	0·165
6	0·168	0·2	0·198

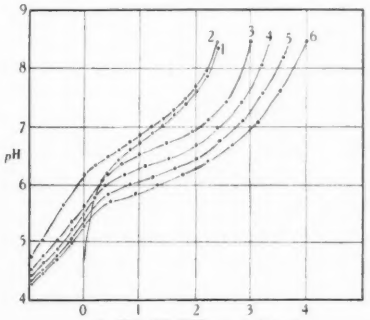

Fig. 5.

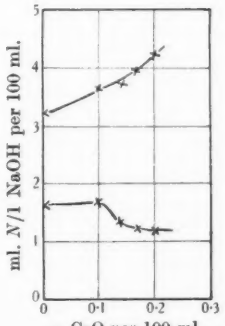

Fig. 6.

lowered. This leads to a diminishing titration value between *p*H 6·6 and 8·0 with increasing calcium concentration, as is shown in Fig. 6. In the absence of an excess of calcium the gross titration between these limits is not greatly affected. On the other hand, an excess of calcium, by inducing precipitation at a lower *p*H, causes the titration to decrease with increasing calcium concentration. The phosphate and citrate concentrations were chosen in this experiment to be normal for milk, and a typical calcium concentration would be 0·165 g. CaO per 100 ml., so that it would appear that the titratable acidity of fresh milk should vary inversely with the calcium concentration.

The titration between *p*H 4·8 and 8·0 shows that the quantity of sodium hydroxide needed increases with the calcium concentration, since the precipitation of calcium phosphate occurs within these limits irrespective of the

concentration of citrate. The titration value of sour milk would increase with increase of calcium concentration.

It may again be emphasised here that these curves do not represent true equilibria but are meant to approach the conditions obtained in actual titrations. The results vary greatly with the time between addition of reagent and measurement of *p*H, and it is not safe to draw any but broad conclusions from them. No attempt has been made to analyse the curves more exactly.

The titration curves of natural and synthetic acid whey.

In order to see how such curves compare with titrations of natural milk solutions a comparison has been made of the titration of phosphate-citrate-calcium solutions and acid wheys.

The titration curves of two samples of acid whey are shown in Fig. 7. These wheys were prepared by acidifying fresh milk with hydrochloric acid at the rate of 6 ml. of 2*N* acid per 250 ml. of milk. This precipitated all the casein, which was filtered off. The resultant whey was analysed and used for the titration. The constituents determined by analysis were CaO, MgO, P_2O_5 and citric acid, the results being as follows:

	Sample 1 g. per 100 ml.	Sample 2 g. per 100 ml.
CaO	0·171	0·220
MgO	0·020	0·021
P_2O_5	0·206	0·165
Citric acid	0·180	0·140

Sample 1 was from the mixed milk of a herd of cows and sample 2 from the milk of a cow nearing the end of the lactation period. The latter was rather abnormal in composition and had a low titratable acidity.

Solutions of the same composition as these wheys in regard to CaO, MgO, P_2O_5 and citric acid were prepared from solutions of the salts as described in the previous work, and the *p*H adjusted with hydrochloric acid to that of the acid whey. The magnesium was added in the form of magnesium sulphate. The effect of magnesium on the titration of such solutions was not investigated because it occurs in such comparatively small proportions in milk. Doubtless it has some slight modifying action on the titration curve, since, like calcium, it forms an insoluble phosphate.

Fig. 7 shows that the acid whey from milk having the higher phosphate and citrate and lower calcium concentration has a higher titratable acidity (from *p*H 4·8 and 6·6 to 8·0) than the whey from sample 2, as would be expected from the work already described. The "synthetic" wheys which are plotted in broken lines were found to have titration curves in close agreement with the wheys prepared from milk. The conclusions drawn from the titration curves of these salt solutions therefore appear to be justified when applied to natural milk products of similar compositions.

The titration of acid whey is of particular importance in the manufacture of cheese, where the chief control over the various stages of manufacture is

effected by the titration of the whey. The casein is first precipitated by rennin. As manufacture proceeds acidity is developed by fermentation and the acid whey expressed from the curd is titrated. The extra acidity is due principally to lactic acid. In order to compare the effects of lactic and hydrochloric acids on the acidity of the whey two samples of whey were titrated in which the casein was precipitated by lactic acid produced by natural fermentation.

These samples were prepared by inoculating the milk with a pure strain of *Str. lactis* known to produce a particularly clean souring with a negligible production of volatile acids and carbon dioxide. Souring was allowed to proceed until a firm curd was produced, when the whey was drawn off. This whey was analysed in the same manner as the hydrochloric acid wheys, and

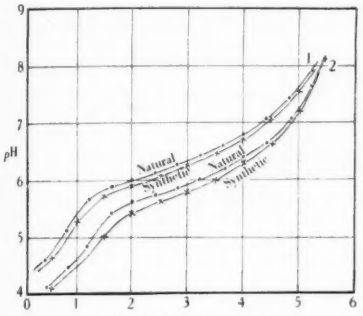

Fig. 7.

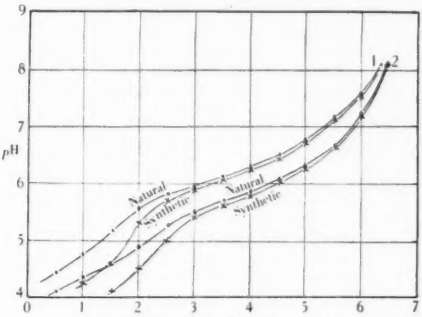

Fig. 8.

the whey and synthetic mixtures of similar composition were titrated. The milk used was from the same source as that used for the hydrochloric acid whey experiments although drawn two days later. The analyses show the composition of the two types of whey to be very similar, the chief difference being the substitution of lactates for chlorides. The compositions of the naturally soured wheys were as follows:

	Sample 1 g. per 100 ml.	Sample 2 g. per 100 ml.
CaO	0·169	0·217
MgO	0·018	0·018
P_2O_5	0·200	0·163
Citric acid	0·175	0·137

Fig. 8 shows the titration curves of these "*self-sour*" wheys compared with salt mixtures of the same concentrations but acidified with hydrochloric acid. Again the agreement is good. The curves are similar to those for the hydrochloric acid wheys for the region from *p*H 4·8 to 8·0. This is to be expected from the dissociation constant of lactic acid, $pK = 3{\cdot}8$, as at *p*H 4·8 it would be 90 per cent. neutralised and its buffer action would be almost negligible. Below *p*H 4·8 the curves flatten out as the buffering effect of the lactic acid becomes operative.

The titration of solutions containing casein, phosphates, citrates and calcium.

The only other important buffer constituent of milk which remains to be studied is the casein, and from its properties one would expect it to have a marked effect on the titration curve of the milk constituents.

The titration curves of pure casein with sodium hydroxide, calcium hydroxide and hydrochloric acid have been studied by several workers, but it will be sufficient to refer to the results of Palmer and Richardson (14). They show that, when titrated in pure solutions, the protein exerts little buffering about its isoelectric point. On the alkaline side maximum buffering occurs between *p*H 6 and 7 and then decreases until it is slight at 8. With addition of acid buffering is slight until a *p*H of 3·2 is reached.

At any given *p*H the equivalents of sodium or calcium hydroxide bound by the protein are not identical, as shown by the following figures taken from Palmer and Richardson's results:

	Base bound per g. of casein g. equivalents $\times 10^{-5}$	
*p*H	NaOH	$Ca(OH)_2$
6	8	32
7	58	70
8	76	88

Thus in milk, where calcium is present, a larger quantity of base might be required to bring the casein to *p*H 8 than would be expected from the sodium hydroxide titration curve.

Wright (15) found by measuring the distribution of calcium about a semi-permeable membrane, on one side of which was a solution containing casein, and applying Donnan's theory of membrane equilibrium to his results, that calcium caseinate was not highly dissociated. The degree of dissociation was approximately 0·25 at the concentration of casein commonly occurring in milk. Because of this slight dissociation casein would be expected to have a similar effect to citrate in delaying the precipitation of calcium phosphate, thus bringing the *p*H at which this occurs (and consequently the maximum buffering of milk) to a higher value.

In the following experiments a series of solutions of phosphate, citrate and calcium, in concentrations typical of milk and containing various proportions of casein, were titrated.

The casein used was Kahlbaum's casein "nach Hammerstein." 20 g. of this were dissolved by grinding with 120 ml. *N*/10 sodium hydroxide and making to 200 ml. with distilled water. The solution was complete within 10 min. and was mixed with the other solutions within another 5 min., so that any racemisation of the casein by the alkali was negligible, particularly as the *p*H of the solution did not exceed 8. Various quantities of this casein solution were added to the phosphate-citrate-calcium solution and the mixtures

allowed to stand 2 hours before commencing the titration. The nitrogen content of the original casein solution was determined and the casein content of the mixtures calculated from this figure. The compositions of the various solutions titrated are given in Table IV.

Table IV.

No.	P_2O_5	CaO	Citric acid	Casein
1	0·168	0·163	0·2	0
2	0·168	0·163	0·2	1·08
3	0·168	0·163	0·2	1·62
4	0·168	0·163	0·2	2·16
5	0·168	0·163	0·2	2·70

The results, together with a titration curve of milk (curve 6), are shown graphically in Figs. 9 and 10. It should be noted that, owing to the alkalinity of the casein solution, the mixed solutions had increasing initial alkalinities

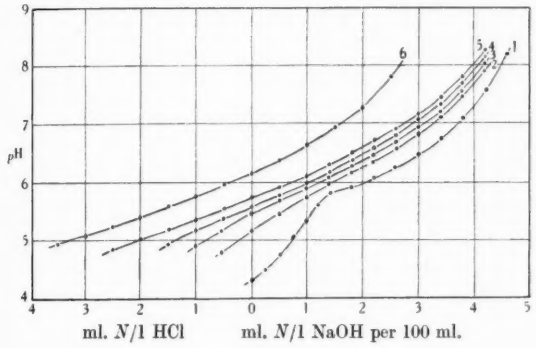

Fig. 9.

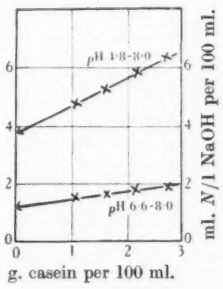

Fig. 10.

with increase of casein concentration, so that when plotted the curves fall on different levels. The point in question is, however, the acid or base necessary to shift the *p*H of each solution between certain fixed levels.

The marked effect which the casein has on the titrations is immediately apparent. When compared with curve 1 all the solutions containing casein show a large increase in buffer value between *p*H 4·8 and 5·8, and a diminution in the intensity at about *p*H 6·0.

Fig. 10 shows that the titration values between *p*H 6·6 and 8·0 increase but slightly (although fairly uniformly) in the region studied with increase of casein concentration. The rate of increase in sodium hydroxide required is approximately 0·2 ml. per g. of casein. Between *p*H 4·8 and 8·0 the titration also increases uniformly with increase of casein and here the effect is larger, the increase being about 0·9 ml. of *N*/1 sodium hydroxide per gram of casein.

These figures, together with the results of Palmer and Richardson with pure casein, are shown in Table V.

Table V.

	Palmer and Richardson's casein titration		ml. $N/1$ NaOH in experimental solutions containing Ca, etc., per g. casein
	ml. $N/1$ NaOH per g. casein	ml. $N/1$ $Ca(OH)_2$ per g. casein	
*p*H			
6·6–8·0	0·43	0·27	0·2
4·8–8·0	0·77	0·88	0·9

From these results it would appear that the response of casein to titration with NaOH is the same as with $Ca(OH)_2$; but the gross change has only been considered over the chosen range of *p*H and the shape of the titration curve cannot be explained on this simple assumption. The subject will be discussed when the buffer curves of these titrations are considered in the next section of this study.

The linear relation between casein concentration and titration agrees with the finding of Harris(5) who claims that casein can be estimated in milk by titrating between two fixed *p*H points. He chooses *p*H 6·65–5·2 and states that the ml. of $N/10$ acid required per 10 ml. of milk are numerically equal to the percentage of protein in the milk. He contends that the ratio of protein to other buffer constituents is constant, but gives no evidence to support this other than the fact that the ratio of protein to ash is fairly constant, as Droop Richmond has pointed out. The ash, however, is a poor indication of the non-protein buffers present, as the citrates are not included though they are believed to vary to a larger extent than the other constituents.

The titration curve of milk shown in Fig. 9 is quite similar to curve 4. This and other titrations will be considered in more detail in the section on the buffer curves.

Conclusions from the titration curves of solutions of milk constituents.

The conclusions which have been arrived at are summarised in Table VI.

Table VI.

	Effect of increasing concentration on the titration with sodium hydroxide	
Constituent	Between *p*H 4·8 and 8·0	Between *p*H 6·6 and 8·0
Phosphate	Increases the titration	Increases the titration
Citrate	Very little effect on titration	Increases the titration
Calcium	Increases the titration	Slightly decreases the titration
Casein	Increases the titration	Slightly increases the titration

The complexity of the reactions involved and the fact that closely reproducible results can only be obtained when the titrations are performed in exactly the same manner make it appear futile to attempt to specify quantitatively the above results. However, the curves given show the order of magnitude of the changes in the titration caused by the main buffer constituents of milk when titrated to the phenolphthalein end-point from conditions corresponding to fresh and sour milk.

The experiments have shown how each of the constituents has a decided effect on the equilibrium and changes the form of the titration curve in a manner which cannot be predicted from its individual titration curve.

REFERENCES.

(1) VAN SLYKE and BOSWORTH (1914). *Bull. N.Y. St. agric. Exp. Sta.* No. 37.
(2) RICE (1919). *Science*, **50**, 429.
(3) MCINERNEY (1920). *J. Dairy Sci.* **3**, 229.
(4) RICE and MARKLEY (1924). *Ibid.* **7**, 468.
(5) HARRIS (1925). *Proc. roy. Soc.* B, **97**, 364.
(6) WENDT and CLARK (1923). *J. Amer. chem. Soc.* **45**, 881.
(7) HOFFMANN and GORTNER (1925). *J. phys. Chem.* **29**, 769.
(8) HOLT, LA MER and CHOWN (1925). *J. biol. Chem.* **64**, 509.
(9) FARNELL (1926). *J. Soc. chem. Ind., Lond.*, **45**, 343 T.
(10) BRITTON (1927). *J. chem. Soc.* **121**, 614.
(11) HASTINGS and VAN SLYKE (1922). *J. biol. Chem.* **53**, 269.
(12) WILEY (1930). *Biochem. J.* **24**, 856.
(13) WHITTIER (1929). *J. Dairy Sci.* **12**, 405.
(14) PALMER and RICHARDSON (1925). *Third Colloid Symposium Monograph*, p. 112.
(15) WRIGHT (1928). *J. agric. Sci.* **18**, 478.

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637.127.8

95. A STUDY OF THE TITRATABLE ACIDITY OF MILK

II. THE "BUFFER CURVES" OF MILK

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(With 3 Figures.)

The previous section of this study in which the titration curves of various milk constituents were discussed approximately defined their influence on the titration. Some of the conclusions arrived at are amplified and more clearly defined when the buffer index of the curves is considered.

In this discussion Van Slyke's(1) buffer index will be used, *i.e.* dB/dpH, the ratio of the change in normality of acid or base to the corresponding change in *p*H when the change of *p*H is infinitely small. The values recorded in this paper were obtained by measuring the slopes of the corresponding titration curves.

Buchanan and Peterson(2) used this method of expressing the buffer capacity of milk. They found a maximum value of 0·0326 in the region 5·0–5·5 falling to a minimum of 0·00635 between *p*H 8·0 and 8·5. While the general shape of their buffer curve has been confirmed by the following work they draw some rather surprising conclusions from it. They state that there is a great similarity between the titration curves of milk and sodium phosphate and that therefore "phosphates are at least among the most important buffers of milk." While the conclusion is undoubtedly correct, sufficient work has been described in the previous section of this study to show that there is little similarity between the two curves. They also claim that, as the value of the buffer index is not affected greatly near the isoelectric point of the casein, the casein can have little effect on the buffering in this region, *p*H 4·5–5·0. On the other hand, Whittier(3) comes to the opposite conclusion, *i.e.* that the buffer action of the casein is exerted principally between *p*H 4·50 and 5·70 with a maximum at approximately *p*H 5·20. This follows from a consideration of the buffer curves of milk and of the acid whey prepared from it. Whittier considers that the difference between these two curves shows the effect of casein.

Whittier was concerned particularly with the region between *p*H 4 and 7 and did not consider the more alkaline portion of the curves. Moreover, he reached a condition of approximate equilibrium in making the titrations, so that his results are not applicable to the rapid titration of milk to the phenolphthalein end-point. Unless otherwise noted the curves in the following work were obtained from titrations made in the manner described in the previous

paper(4), the value dB/dpH being obtained by measuring the slope of the tangents of the titration curves.

In Fig. 1, curves 1, 2 and 3 are the buffer curves of phosphate, citrate and casein solutions respectively in concentrations typical of milk. Curve 4 is obtained by adding these curves, and the great difference between this and the curve of milk, 5, is apparent. Curve 6, corresponding to the titration of a solution containing calcium in addition to phosphate, citrate and casein, closely follows the curve for milk. The following study indicates the manner in which calcium has such a pronounced effect on the titration.

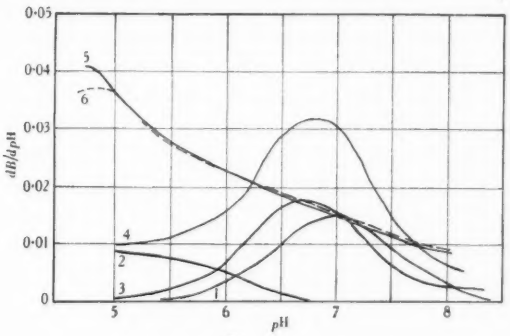

Fig. 1.

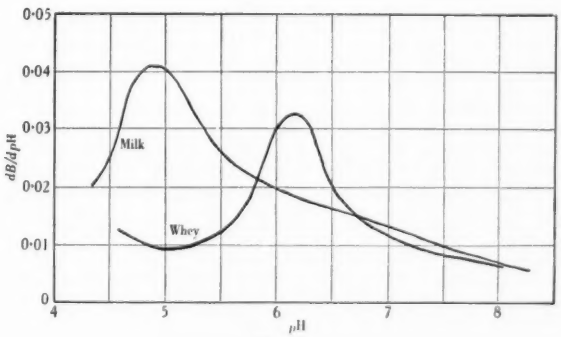

Fig. 2.

Fig. 2 shows the buffer curves of a sample of milk and a sample of whey prepared from it by adding 8 ml. of a $2N$ hydrochloric acid to 250 ml. of the milk and filtering off the precipitated casein. It is known that casein prepared in this way is comparatively pure, and so the titration curve of the whey should show the behaviour of the milk constituents other than the casein. The milk shows a maximum in the curve at pH 5·0, where there is a minimum in the whey curve. At about pH 6·2 the whey has a greater buffering effect than the whole milk, while from pH 6·6 to 8·0 there is not a great difference. The whey curve can be interpreted from the results obtained in the first part

of this work(4). The minimum at *p*H 5·0 shows the slight phosphate and citrate buffering in that region in the presence of calcium. The maximum at *p*H 6·2 is where calcium phosphate is precipitating and tending to hold the *p*H constant.

The curve for milk at *p*H 6·2 is lower than that of the acid whey. In milk calcium phosphate is already present in colloidal solution, since the milk has necessarily been held some time at its normal *p*H, about 6·6. This colloidal calcium phosphate has been investigated particularly by Van Slyke and Bosworth(5) who have shown it to be $CaHPO_4$. Now the work of Holt, La Mer and Chown(6) showed that this phosphate could exist without dissolving at a *p*H as low as 5·0 in solutions of low ionic concentration. It is reasonable, then, to explain the greater buffering of the whey at *p*H 6·2 to the slow precipitation of this phosphate, and part of the increased buffering of the milk at lower *p*H to its solution. Measurement of the area under the curves shows,

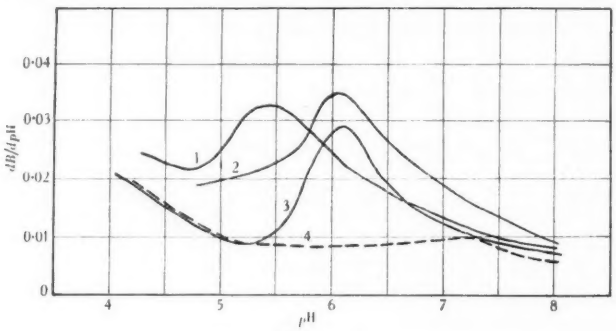

Fig. 3.

however, that this effect can only contribute a proportion of the buffering between 4·5 and 5·8 while, of course, part is attributable to the casein.

Whittier obtained somewhat similar curves except that the maximum buffering of milk occurred at a somewhat higher *p*H, namely 5·5. He considered that the difference between the milk and whey curves represented the buffering of the casein and as this difference curve was so dissimilar to that obtained for simple casein solutions that casein must exist in milk in a form different from that after precipitation.

Fig. 3, curve 1, shows the results of a titration in which the solutions were allowed to stand two hours after addition of the reagents before *p*H measurements were made, as in Whittier's work. The curve for milk agrees closely with his. There is also shown, in curve 2, the results of a back titration of the same milk after adding 3 ml. of 2*N* hydrochloric acid to 100 ml. of the milk but not filtering off the casein. The third curve shows the titration of the acid whey prepared similarly but with the casein filtered off. The difference between the curves of the milk titrated directly and back titrated is immediately apparent. The back titration shows the buffer maximum at about *p*H 6·1, just

where the acid whey curve also has its maximum. Here again the maximum in the buffer curve can be explained as due to the precipitation of the phosphate.

The colloidal calcium phosphate can be removed from milk together with casein by the action of rennin. Rennin whey was prepared from the same milk using rennin which had been dialysed free from salts in a collodion thimble suspended in running water. The buffer curve, 4, of this is also shown in Fig. 3. In the regions *p*H 4·0–5·3 and 7·2–8·0 it is practically identical with that of the acid whey, but between *p*H 5·3 and 7·2 it shows no peak. This again shows the importance of the calcium phosphate in the titration of milk.

From these results it can be definitely stated that the pronounced maximum in the titration curve of milk is partly due to the slow solution and precipitation of calcium phosphate. Briefly recapitulated the evidence is as follows:

When the milk is titrated quickly with acid this maximum occurs at *p*H 5. If two hours elapse before measurement of the *p*H the maximum occurs at *p*H 5·5, as the colloidal calcium phosphate has time to dissolve at a *p*H more nearly approaching its true equilibrium value. If the milk is acidified to an acidity greater than that corresponding to *p*H 5 and then back titrated with alkali the maximum occurs at *p*H 6·1 owing to the delayed phosphate precipitation. This is the same point found with acid whey and solutions of phosphates, citrates and calcium of similar composition, and it has been shown that this maximum is due to precipitation of calcium phosphate. With rennin whey, in which the colloidal calcium phosphate of milk is absent, the maximum in buffer value does not occur.

As the buffer curve is so greatly affected by the method of titration and as the precipitation of calcium phosphate has been shown to affect greatly the form of the curve, Whittier's assumption that the difference between the curve of milk and of acid whey shows the buffer curve of casein does not appear to be justified. His deduction that, because this difference curve is dissimilar to the buffer curve of casein titrated in pure solution, the casein is altered in properties is not upheld by the curves obtained for the titration of buffer solutions containing casein and calcium. Fig. 1 shows the buffer curves of milk and a "synthetic" solution containing 3·0 g. of casein per 100 ml. The similarity in the curves is close and the "synthetic" milk has a maximum at about *p*H 5. At higher *p*H values the buffering is only slightly greater than for milk. It must be noted that this mixture stood for two hours after preparation at about *p*H 6·5 before titration, so that the condition of the salts would approximate that found in milk. In short, this casein "nach Hammerstein," when dissolved with the appropriate salts, behaved in the titration in a similar manner to the casein as it occurs naturally in milk.

The reason for the difference in the curves of milk and acid whey is then the influence of the casein on the calcium phosphate equilibrium and this study of the buffer curves has again emphasised the interdependence of the buffers phosphate, citrate, casein and calcium.

ACKNOWLEDGMENTS.

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REFERENCES.

(1) VAN SLYKE (1922). *J. biol. Chem.* **52**, 525.
(2) BUCHANAN and PETERSON (1927). *J. Dairy Sci.* **10**, 224.
(3) WHITTIER (1929). *J. biol. Chem.* **83**, 79.
(4) WILEY (1935). *J. Dairy Res.* **6**, 72.
(5) VAN SLYKE and BOSWORTH (1915). *J. biol. Chem.* **20**, 138.
(6) HOLT, LA MER and CHOWN (1925). *Ibid.* **64**, 509.

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637.233.12

96. THE NEUTRALISATION OF CREAM FOR BUTTER-MAKING

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(With 4 Figures.)

The practice of neutralising cream which is often received at the factory in a sour condition is now well established, since the production of butter which will keep well in cold storage demands it. Often, however, the acidity of the neutralised cream and hence of the butter is far from that which the butter-maker aimed at by "neutralisation." For instance, the results of an examination of a large number of Victorian butters, quoted by Loftus-Hills, Scharp and Bellair (1), show very large variations in the acidities of butters churned from creams which were supposed to be neutralised to identical acidities. Moreover, while the reaction occurring has been assumed to be a simple neutralisation of lactic acid with the neutraliser used, most of the published papers on the subject show that the acidity of the neutralised cream varies considerably from that desired. Hunziker (2) states that the reduction in acidity with soda neutralisers (carbonate, bicarbonate and sesquicarbonate) corresponds with that calculated from the sodium hydroxide titration of the cream, while with lime the neutralisation is only 80 per cent. of that calculated. The explanation given is that part of the lime combines with the casein to form a calcium caseinate, whereas the soda neutralisers react only with the lactic acid. Hunziker, however, also states that the soda neutralisers do react with the casein. When the erratic results often obtained in practice are considered it is remarkable that, in view of the commercial importance of the subject, neutralisation has never been studied from a more fundamental viewpoint.

Theoretical aspects of cream neutralisation.

It must first be noted that in practice the quantity of alkali to be added for neutralisation is always calculated from the results of a titration with sodium hydroxide and phenolphthalein. The resultant acidity is checked by a similar titration.

For the purposes of discussion the acids and bases present in significant quantities in a sour cream as usually received at the butter factory may be assumed to be present in approximately the proportion given in Table I, which is similar to the table prepared by Clark (3) for fresh milk.

Table I.

	Grams per litre	Equivalents per litre	Mols. per litre			Equivalents of base bound	
Bases:							
K_2O	1·20	0·0255	—	—	—	—	—
Na_2O	0·48	0·0155	—	—	—	—	—
CaO	1·0	0·0357	—	—	—	—	—
MgO	0·13	0·0065	—	—	—	—	—
	Total	0·0832					
Acids:							
Lactic	2·90	—	0·0322	as $C_3H_5O_3'$	0·0293	0·0293	0·0293
P_2O_5	1·00	—	0·141 (H_3PO_4)	H_2PO_4'	0·0141	0·0141	0·0141
Citric	1·20	—	0·00625 ($H_3C_6H_5O_7$)	$C_6H_5O_7'''$	0·00037	0·0011	as $C_6H_5O_7'''$
				$HC_6H_5O_7''$	0·00363	0·0073	0·0188
				$H_2C_6H_5O_7'$	0·00225	0·0023	—
Cl	0·70	—	0·0198	—	—	0·0198	0·0198
CO_2	0·12	—	—	—	—	—	—
SO_3	0·07	—	0·0009	—	—	0·0009	0·0009
Protein chiefly casein	25·0	—	—	—	—	—	—
					Total	0·0748	0·0829

Dissociation constants:

	pK_1	pK_2	pK_3
Carbonic	6·5	10·2	—
Lactic	3·8	—	—
Citric	3·0	4·6	5·8
Phosphoric	1·97	6·9	11·6

pH of cream = 4·8. Titratable acidity, 0·40 per cent.

The above figures have been calculated for a cream containing 40 per cent. fat, obtained from typical milk. A pure lactic fermentation of the lactose has been assumed, the citrate not being attacked. In practice considerable variations from this are possible, but the figures may be used as a basis for discussion and the effects sometimes observed explained from them without having to assume any abnormal bacterial action.

From a consideration of the dissociation constants and the equation

$$p\mathrm{H} = pKa + \log \frac{\text{salt}}{\text{acid}},$$

the following conclusions are reached.

The lactic acid at pH 4·8 would be about 90 per cent. neutralised. The phosphoric acid would be present as dihydrogen phosphate. The hydrochloric and sulphuric acids would be present completely as salts, and the carbonic acid as free acid. The casein is very close to the isoelectric point and would bind a negligible proportion of base. The citric acid when calculated according to the above equation gives the proportions of mono-, di- and tribasic acids shown in Table I. The equation is not, however, applicable where the three dissociation constants are so close, and in the present instance is incorrect, as it depends on the assumption that the salts formed are completely dissociated, whereas calcium citrate is but slightly dissociated. The experiment described in a previous paper(6) shows that, at this pH and in the presence

of calcium, the citric acid is completely neutralised. If the citric acid is all calculated to the tribasic form, the figures in the last column are obtained and a close agreement found between the equivalents of acid and base present.

A previous paper (6) has shown the results obtained when such solutions are neutralised to *p*H 8 with sodium hydroxide. It has also been shown that an increase in the concentration of calcium causes an increase in the titration between *p*H 4·8 and 8·0 and *p*H 4·8 and 6·6. The maximum in the buffer curve was found to be due to the precipitation of calcium phosphate, and this was a comparatively slow reaction causing the *p*H to fall for a period of about two hours after addition of the alkali.

Hence, if lime were used as a neutralising agent the expected reduction in acidity (as deduced from the sodium hydroxide titration) would not be obtained because the increased calcium concentration would cause an increased basic phosphate precipitation. Moreover, the titration curves of casein obtained by Palmer and Richardson (4) indicate a greater binding of calcium than of sodium, although as the work on the buffer index has shown, their figures cannot be applied directly to milk.

When sodium bicarbonate is used as a neutraliser it is obvious that the full reduction in acidity cannot be obtained before the cream is pasteurised. Sodium bicarbonate is neutral to phenolphthalein, so that, in the cold, complete neutralisation of the cream to this indicator could never be obtained. When the cream is pasteurised carbon dioxide is lost and the reaction more nearly approaches that obtained in the titration with sodium hydroxide. When the holding system of pasteurisation is used, however, the loss of carbon dioxide is probably never complete and the calculated reduction in acidity is not obtained. The carbon dioxide is more completely driven off in the usual flash pasteurisation, so that a lower acidity is found in the cream.

The carbon dioxide originally present in the cream behaves in the same way. If the sample which is titrated is not boiled this carbon dioxide is included in the acidity estimation, but after pasteurisation of the main bulk of cream the carbon dioxide is driven off and the neutralisation obtained may be greater than was expected. It has been suggested that other volatile acids produced by bacterial action sometimes occur in cream and in a similar way lead to errors in neutralisation. This is hardly probable, however, as the only acids likely to be found are not only much less volatile than carbon dioxide, but are considerably stronger, and at the *p*H at which the cream is pasteurised they would be held back as salts.

EXPERIMENTAL WORK ON CREAM NEUTRALISATION.

The only neutralisers used for experiment were milk of lime and sodium bicarbonate solution. Probably over 70 per cent. of Australian butter factories use sodium bicarbonate and a considerable proportion of the remainder use lime. A few employ proprietary neutralising agents which are usually either

sodium sesquicarbonate or mixtures of sodium carbonate and bicarbonate. The general results with such substances would, from the point of view of the present work, be similar to those obtained with sodium bicarbonate.

The sodium bicarbonate solution used was of such strength that 1 ml. was equivalent to 0·110 g. of lactic acid (1·22*N*).

The milk of lime was prepared from chemically pure, freshly ignited, calcium oxide. Analysis showed that carbonates and magnesium were absent. One millilitre of this was equivalent to 0·101 g. of lactic acid (1·12*N*).

For convenience milk was used in most of the experiments rather than cream. The aqueous phases of milk and cream, which are concerned with the neutralisation reactions, are of the same composition, so that results obtained with milk are applicable to cream when allowance is made for the volume of inert fat. Admittedly there may be minor differences due to the protein adsorbed on the fat globules, which may react with alkali, but a few experiments in which cream was used showed this effect to be slight.

The general plan of experiment was as follows:

About 1·5 litres of fresh milk were inoculated with a starter known to produce a particularly clean lactic-acid fermentation. When an acidity of 0·60 per cent. (as lactic acid) had developed, 50 ml. portions were pipetted into a series of 200 ml. conical beakers. An acidity of 0·60 per cent. in the milk corresponds to approximately 0·40 per cent. in a cream of 35 per cent. fat content, which is typical of Australian butter-factory cream. To the beakers containing 50 ml. of cream the appropriate quantities of $Ca(OH)_2$ or $NaHCO_3$ solutions were added and the mixtures thoroughly agitated at 15° C. After 15 min. 10 ml. were taken for titration with sodium hydroxide and 5 ml. for *p*H determination by the quinhydrone method. The remaining 35 ml. were pasteurised; the holding system was imitated by heating to 145° F. for 30 min. in a water bath and cooling, and the flash system by heating rapidly to the desired temperature over a hot gauze with constant stirring and immediately cooling. The smallness of the sample ensured rapid heating, 180° F. being reached in 80 sec. The results should approach those obtained in practice, but it is recognised that the imitation of the typical commercial flash pasteurisation is not close. The flash type of pasteuriser in which the cream flows in a thin film over a heated dome would probably allow a greater escape of carbon dioxide than this laboratory method. When the cream is deodorised by boiling *in vacuo* the escape of carbon dioxide is probably quantitative.

A rapid electrometric titration of 100 ml. of the milk with *N*/1 sodium hydroxide was also made in each series of experiments.

Thus the results obtained gave the titratable acidity and *p*H, both before and after pasteurisation, of a series of milks to which known quantities of neutraliser had been added.

RESULTS WITH LIME.

The results of the first series of experiments, in which lime was used as a neutraliser and in which flash pasteurisation was employed, are given in Table II.

Table II.

ml. milk of lime added to 50 ml. sour milk	Before pasteurisation		After flash pasteurisation at 180° F.	
	Titratable acidity %	*p*H	Titratable acidity %	*p*H
0	0·60	4·83	—	—
0·9	0·44	5·53	0·46	5·55
1·6	0·36	5·83	0·38	5·81
2·05	0·25	6·13	0·23	6·08
2·30	0·19	6·32	0·19	6·36
2·55	0·155	6·49	0·14	6·47
2·80	0·096	6·87	0·08	6·84
3·05	0·06	7·25	0·05	7·27
3·55	Alkaline	8·70	Alkaline	8·31

On pasteurisation the samples coagulated, but a thorough agitation broke up the curd and permitted a representative portion to be taken for analysis. It will be noted that pasteurisation had only a slight effect on the titratable acidity and *p*H.

From these results Table III has been calculated:

Table III.

Gram equivalents base $\times 10^{-3}$ per 100 ml. milk	*p*H	Reduction in titratable acidity	Theoretical reduction in acidity	Difference between actual and theoretical reduction	Percentage of theoretical reduction obtained
0	4·83	0	0	—	—
2·02	5·53	0·16	0·18	0·05	70
3·57	5·83	0·24	0·33	0·12	73
4·59	6·13	0·35	0·42	0·07	83
5·12	6·32	0·41	0·48	0·07	85
5·69	6·49	0·445	0·52	0·075	86
6·23	6·87	0·504	0·57	0·066	88
6·80	7·25	0·54	0·61	0·07	88
7·95	8·86	Alkaline	0·72	—	—

The theoretical reduction in acidity was calculated as shown in the following example:

To 50 ml. of milk of acidity 0·60 per cent. were added 2·8 ml. $Ca(OH)_2$, equivalent to $2{\cdot}8 \times 0{\cdot}101 = 0{\cdot}283$ g. lactic acid.

Therefore $\frac{0{\cdot}60}{2} - 0{\cdot}283 = 0{\cdot}017$ g. of lactic acid remain unneutralised.

52·8 ml. milk + neutraliser contain 0·017 g. lactic acid.

Therefore acidity $= \frac{100 \times 0{\cdot}017}{52{\cdot}8} = 0{\cdot}032$ per cent. and reduction $= 0{\cdot}60 - 0{\cdot}032 = 0{\cdot}57$ per cent. lactic acid.

The convention of calculating the titratable acidity to lactic acid has of course been followed.

The significance of these results is more apparent when they are shown graphically. Fig. 1 shows the relationship between added alkali and *p*H. The curve for a sodium hydroxide titration is also shown.

The addition of lime brings the milk to some definite *p*H on the $Ca(OH)_2$ curve. The acidity is then determined by titrating from this *p*H with sodium hydroxide, but the titration for the milk measured from this *p*H cannot be predicted from the sodium hydroxide curve. This is because calcium has been added to the solution and it has already been shown that this modifies the sodium hydroxide titration. With increasing quantities of calcium the subsequent NaOH titration will tend to follow the $Ca(OH)_2$ titration curve.

The $Ca(OH)_2$ curve shows a decided increase over the NaOH curve in buffering between about *p*H 5·6 and 6·2. Previous work has shown that this

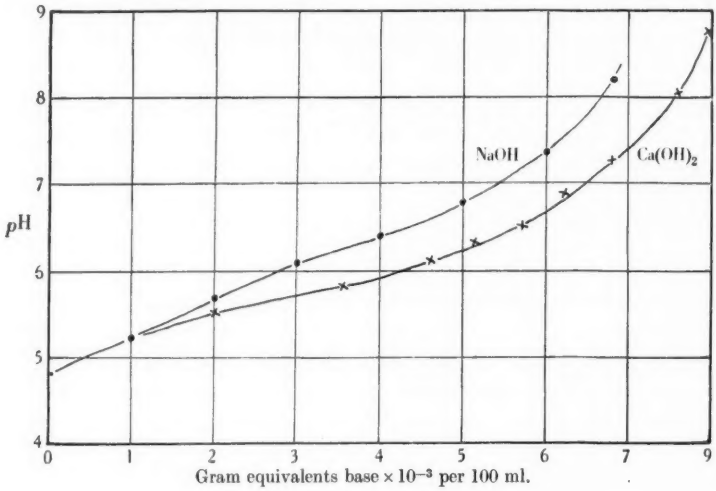

Fig. 1.

is due to the precipitation of calcium phosphate. Above *p*H 6·2 the two curves run approximately parallel, so that above this *p*H the reduction in titratable acidity caused by lime would be expected to be approximately a constant quantity less than the theoretical reduction by an amount equivalent to the difference between the quantity of NaOH and $Ca(OH)_2$ required to titrate to the phenolphthalein end-point; in this case $0{\cdot}8 \times 10^{-3}$ g. equivalents per 100 ml. equivalent to 0·07 per cent. lactic acid. The experimental results show this to be so.

When $3{\cdot}57 \times 10^{-3}$ g. equivalents of $Ca(OH)_2$ had been added the *p*H was 5·83 and the reduction in acidity only 0·24 per cent. At this point the increased buffering due to calcium phosphate precipitation is at a maximum. If it is assumed that the NaOH titration will be similar to that for the $Ca(OH)_2$ from this point on, the curve shows that approximately 4·2 g. equivalents of

base per 100 ml. will be required to bring the solution to the phenolphthalein end-point. This is equivalent to 0·38 per cent. lactic acid or a reduction in acidity of 0·60 − 0·38 = 0·22 per cent. The figure actually found, *i.e.* 0·24 per cent., is reasonably close to this.

A second series of experiments was made in which the milk, after neutralisation, was pasteurised by heating to 145° F. for 30 min. The results confirmed those already described, but since no new points arose they are not described in detail. Owing to the small effect on the neutralisation with lime which was caused by pasteurisation it was improbable that changes in the method of pasteurisation would give rise to appreciable differences.

A further series of experiments was made with a sample of sour cream from the same source as the milk used previously. The same starter as before was used and an acidity of 0·40 per cent. developed. The cream was not pasteurised after neutralisation but was allowed to stand for 15 min. after addition of the lime. The results are shown in Table IV.

Table IV.

Fat 34·5%. Acidity 0·40%.

Gram equivalents $Ca(OH)_2$ added per 100 g. of cream	*p*H	Reduction in titratable acidity	Theoretical reduction in titratable acidity	Difference between actual and theoretical reduction	Percentage of theoretical reduction obtained
0	4·90	0	0	—	—
1·0	5·41	0·08	0·09	0·01	89
1·5	5·65	0·11	0·135	0·025	81
2·0	5·76	0·135	0·18	0·045	75
2·5	5·91	0·17	0·225	0·055	75
3·0	6·04	0·22	0·27	0·05	81
3·5	6·23	0·26	0·315	0·055	82
4·0	6·59	0·31	0·36	0·05	86
4·5	7·05	0·35	0·405	0·055	86

These results confirm, in general, those obtained with milk. When the cream is neutralised with lime to a *p*H of about 5·8 a minimum percentage (about 75) of the theoretical reduction in acidity is obtained. Above and below this figure a greater reduction is obtained.

The acidity at which different factories churn their cream generally varies between 0·10 and 0·25 per cent. With the above cream these figures would correspond to *p*H 6·6 and 5·85 and the percentage of the theoretical reduction obtained on neutralising would be between 75 and 86. Hunziker's(2) recommendation to calculate on an 80 per cent. reduction is therefore confirmed and extended by the above results. It would probably be impracticable to attempt to calculate the reduction more closely, for it must be remembered that the present work has been done on milk and cream in which there had been a particularly clean lactic fermentation. When an appreciable quantity of carbon dioxide is formed in souring it is lost in pasteurisation and a reduction in the titratable acidity results. In any case the titration of milk and cream with sodium hydroxide is not particularly easy to make with exactitude and, at the butter factory, very close control of acidity is impossible when titration is carried out in the usual manner.

RESULTS WITH SODIUM BICARBONATE.

Table V shows the results of a series of experiments with sodium bicarbonate. The method was the same as when lime was used.

Table V.

ml. $NaHCO_3$ solution added to 50 ml. milk	Before pasteurisation		After flash pasteurisation at 180° F.	
	Titratable acidity %	*p*H	Titratable acidity %	*p*H
0	0·57	4·89	0·56	4·90
0·80	0·42	5·65	0·40	5·72
1·30	0·39	5·99	0·29	6·02
1·70	0·35	6·13	0·19	6·40
1·95	0·33	6·23	0·13	6·63
2·20	0·315	6·27	0·105	6·83
2·35	0·305	6·32	0·10	6·95
2·60	0·30	6·42	0·09	7·10
3·05	0·29	6·47	0·05	7·39

Table VI has been constructed from the results *before* flash pasteurisation.

Table VI

Gram-equivalents $\times 10^{-3}$ of $NaHCO_3$ per 100 ml. milk	*p*H	Reduction in titratable acidity	Theoretical reduction in acidity	Difference between actual and theoretical reduction	Percentage of theoretical reduction obtained
0	4·89	0	0	—	—
1·95	5·69	0·15	0·17	0·02	88
3·17	5·99	0·18	0·29	0·13	55
4·14	6·13	0·22	0·38	0·16	57
4·75	6·23	0·24	0·44	0·20	54
5·37	6·27	0·255	0·49	0·235	52
5·71	6·32	0·265	0·52	0·255	51
6·32	6·42	0·27	0·57	0·30	47
7·42	6·47	0·28	0·67	0·39	42

The first addition of bicarbonate causes a fall, approaching the theoretical, in titratable acidity and a large increase in *p*H. Larger quantities of bicarbonate, however, cause far from quantitative reductions in acidity and only a very gradual rise in *p*H. These effects are caused by retention of carbon dioxide in the solution and will be discussed more fully when further experimental results are considered.

The results *after* flash pasteurisation are given in Table VII.

Table VII.

Gram equivalents $\times 10^{-3}$ of $NaHCO_3$ per 100 ml. milk	*p*H	Reduction in titratable acidity	Theoretical reduction in acidity	Difference between actual and theoretical reduction	Percentage of theoretical reduction obtained
0	4·90	0	0	—	—
1·95	5·71	0·17	0·17	0	100
3·17	6·02	0·28	0·29	0·01	97
4·14	6·40	0·38	0·38	0	100
4·75	6·63	0·44	0·44	0	100
5·37	6·83	0·465	0·49	0·025	95
5·71	6·95	0·47	0·52	0·05	90
6·32	7·10	0·48	0·57	0·09	84
7·42	7·39	0·52	0·67	0·15	78

The marked difference caused by pasteurisation and loss of carbon dioxide is apparent.

Further experiments were made with milk from the same source which had been soured to the same extent. In one series the milk after neutralisation was holder pasteurised by heating to 145° F. for 30 min., and in another series was boiled for two minutes after neutralising.

The results of these tests together with those already described are given in Figs. 2 and 3. Fig. 2 shows the relationship between added bicarbonate and *p*H and a titration curve of 100 ml. of the milk with *N*/1 sodium hydroxide. Fig. 3 shows the reduction in titratable acidity obtained with different quantities of bicarbonate and the theoretical reduction.

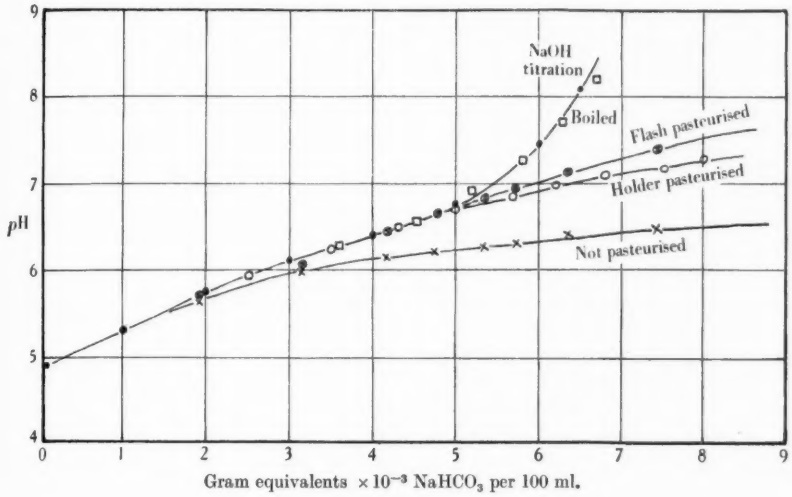

Fig. 2.

The effect of carbon dioxide in the solution is well shown in these curves. Two factors influence the quantity of carbon dioxide retained and hence the degree of neutralisation, namely, pasteurisation and the alkalinity of the solution. The greater the degree of neutralisation aimed at the greater is the amount of carbon dioxide that has to be lost and the more alkaline is the solution.

When the solution was boiled the calculated reduction in acidity was obtained. With flash pasteurisation there was complete neutralisation until the milk had an acidity of about 0·2 per cent. as lactic acid. At lower acidities the neutralisation was not as great as calculated. With the holder method of pasteurisation neutralisation at the lower acidities was slightly less than with the flash system but was practically complete above an acidity of 0·2 per cent. When the milk was not pasteurised, neutralisation was far from

complete, the reduction in titratable acidity being, at low acidities, less than 50 per cent. of the theoretical.

The amount of carbon dioxide lost in factory operation will depend largely upon the design of the pasteuriser. With the usual flash pasteuriser and surface cooler the loss will probably be nearly complete and the calculated neutralisation will be obtained. Where the cream is pasteurised by the holder system, however, and where a low acidity is aimed at, this result will not be obtained. The usual holder pasteuriser gently agitates the cream in bulk while it is maintained at 145° F. and the cream is cooled in the same container. Very often the container is covered during the whole of the operation, so that the loss of carbon dioxide would be retarded.

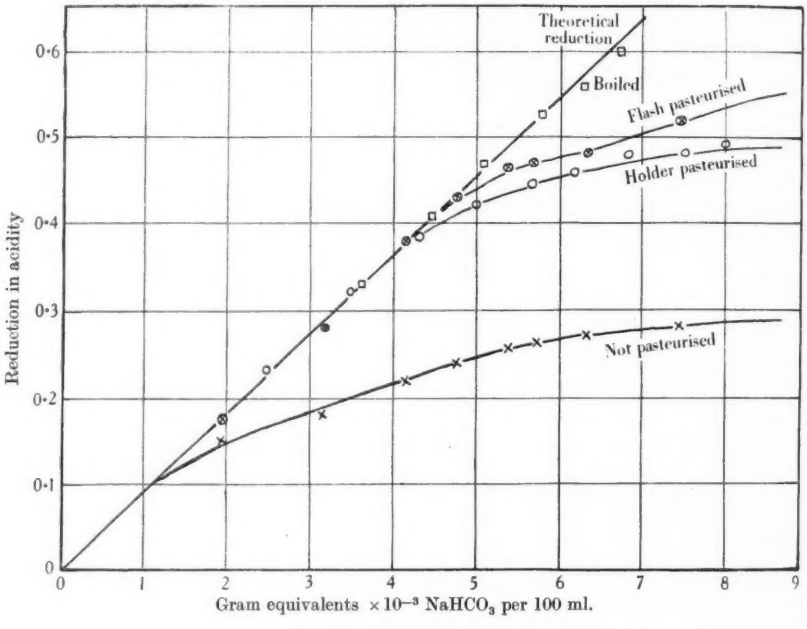

Fig. 3.

THE TITRATABLE ACIDITY AND *p*H OF CREAMS NEUTRALISED WITH LIME AND SODIUM BICARBONATE.

The acidity of the cream is of great importance in determining the keeping quality of butter in cold storage and is always controlled by titration. The reason why acidity plays such a large part in butter deterioration is not definitely established but it is certainly not a specific effect of lactic acid, for Rogers and Gray (5) showed that hydrochloric and acetic acids cause similar changes. One would expect by analogy with enzyme and catalytic action that it would be the intensity factor rather than the quantity factor of acidity

which would be of importance, *i.e.* *p*H rather than titratable acidity. If this is correct it is important to know whether, when a cream is neutralised to a given titratable acidity, there will be any marked difference in *p*H if different neutralisers are used.

The experiments in which lime was used as a neutraliser showed the difference in the titration curves of cream with sodium hydroxide and lime, and it was found that above a *p*H of about 6·2 the curves were parallel. The earlier work on the titratable acidity of milk showed that above *p*H 6·5 calcium had but a slight effect on the titration. When the *p*H was lower than 6·2 it increased the titration, owing to the precipitation of calcium phosphate. With a comparatively large quantity of calcium present, however, when neutralising with lime, precipitation of the phosphate occurred at *p*H 5·6 and a subsequent titration with sodium hydroxide from above this *p*H might not

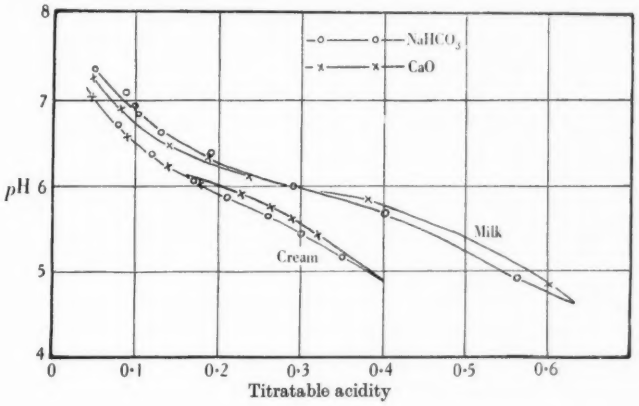

Fig. 4.

differ materially from that of a cream without the lime. Below this *p*H with cream of the typical acidity being considered (0·4 per cent.), the neutralisation is being commenced and a large difference between different neutralisers could not be expected. One would expect then, that in the lower range, *p*H 5 to 6, the titratable acidity would be somewhat higher for a given *p*H when using lime for neutralisation than when using sodium hydroxide, but the effect would not be large.

With sodium bicarbonate the neutralisation curve was practically identical with the sodium hydroxide curve except when carbon dioxide was retained in solution. As both lime and sodium bicarbonate therefore should give results not differing greatly from sodium hydroxide the relation between *p*H and titratable acidity of cream neutralised with the two reagents would be expected to be quite close.

The results of the experiments already described confirm this conclusion. Fig. 4 shows the results obtained with lime and sodium bicarbonate after

flash pasteurisation when milk was neutralised. The results of another series of experiments with cream are also shown. For these the same cream was neutralised to various acidities with lime and bicarbonate and then flash pasteurised. At the higher acidities the *p*H is slightly higher (0·1 unit) for a given titratable acidity when lime is the neutraliser. Such wide variations occur in the *p*H and titratable acidity of Australian butters that differences due to different neutralisers appear insignificant.

SUMMARY.

The reactions which occur when sour cream is "neutralised" with calcium hydroxide and sodium bicarbonate are discussed from the point of view of the acid-base equilibria.

Experimental results are recorded showing that:

1. When lime is the neutraliser, pasteurisation has little effect on the *p*H or titratable acidity of the neutralised cream.

2. The reduction in acidity when lime is used is shown to vary from that calculated from the sodium hydroxide titration to an extent which depends on the *p*H of the neutralised cream. The reasons for and extent of this variation are defined.

3. When sodium bicarbonate is used as a neutraliser the loss of carbon dioxide (which is influenced by the method of pasteurisation adopted) has most influence on the degree of neutralisation obtained and the experiments show the extent of this at various acidities.

The variations in the *p*H-titratable acidity relationship of creams neutralised with calcium hydroxide and sodium bicarbonate are defined.

REFERENCES.

(1) LOFTUS-HILLS, SCHARP and BELLAIR (1934). *J. Dairy Res.* **5**, 133.
(2) HUNZIKER (1927). *The Butter Industry.* 2nd ed. Chicago.
(3) CLARK (1928). Rogers' *Fundamentals of Dairy Science.* New York: Chemical Catalogue Co.
(4) PALMER and RICHARDSON (1925). *Third Colloid Symposium Monograph*, p. 112.
(5) ROGERS and GRAY (1909). *Bull. U.S. Bur. Anim. Ind.* No. 114.
(6) WILEY (1934). *J. Dairy Res.* **6**, 72.

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BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION F. MILK-BORNE DISEASES.

I. STREPTOCOCCAL INFECTIONS.
II. *BRUCELLA* INFECTIONS.
III. *SALMONELLA* AND DYSENTERY INFECTIONS.
IV. TUBERCULOSIS.

THE important diseases in this category are:

(1) the streptococcal infections, including scarlatina, tonsillitis and their sequelae;

(2) the *Brucella* infections, chiefly undulant fever;

(3) the enteric group, including continued fever, due to the typhoid bacillus and other salmonellas, and acute gastro-enteritis or colitis, due to salmonellas or dysentery bacilli; and

(4) tuberculosis in all its manifestations.

Improved methods of identifying bacterial species have greatly increased the assurance with which such human infections can be ascribed to specific infection of cows or their milk. The following notes deal chiefly with these technical advances.

I. THE STREPTOCOCCAL INFECTIONS.

Differentiation of the streptococci found in the healthy and inflamed udder and in cows' milk and its derivatives has long been difficult and doubtful. The well-known studies of their morphology and cultural behaviour by Orla-Jensen, Alice Evans, S. H. Ayers, W. D. Frost and G. J. Hucker have been followed by serological analysis of the haemolytic group, and this analysis, as shown especially by R. C. Lancefield (1) and by F. C. Minett and A. W. Stableforth (2), distinguishes satisfactorily the streptococci ordinarily responsible for human disease from those found only in the infections of bovine and other animals. The haemolytic streptococci, as the name implies, are identified by their ability to cause lysis of red blood corpuscles in culture media, so that their colonies on nutrient agar containing blood are surrounded by a zone of clarification. A good deal of confusion still exists, however, as to what constitutes the typical behaviour of a haemolytic streptococcus in this respect, since the degree of clarification and the character of the zone depend on the species of animal whose blood is used as indicator and on the position of the colony in the medium, *i.e.* whether it is in the depths or on the surface of the blood-agar mixture. The use of horse-blood agar (5 per cent. whipped or oxalated) as a thin film of blood agar on top of a layer of ordinary nutrient agar (Gordon's device) and the inoculation of this medium on the surface only, have been recommended by F. Griffith (3) as the standard medium and method for the detection of the various differential features, and especially of alpha haemolysis, beta haemolysis, etc., as first described by Brown (4). The blood of other animals, especially rabbits and sheep, is unsuitable owing to pronounced differences in lysability of the corpuscles from different individuals. Deep colonies, such as are obtained by inoculation of the fluid blood agar, fail to show differences in the character of the zone (easily perceptible with surface colonies) and in the form and consistency of the colony itself, both important differential features.

When grown in the manner prescribed by Griffith (3), colonies of the haemolytic streptococci responsible for human disease are surrounded, after 24 hours' incubation at 37° C., by a clear zone in which practically all the red cells suspended in the agar mesh have dissolved, and in which their haemoglobin has diffused without undergoing chemical alteration, *i.e.* beta haemolysis. The colonies themselves are variable in shape, surface and consistency, but the range of variation is limited and readily learnt by experience. Griffith (5) proposes that the specific name, *Streptococcus pyogenes*, should be confined to streptococci producing colonies within this range of variation, showing the typical haemolysis, and producing disease in man. Haemolytic streptococci are found in the cow's udder, chiefly when some degree of mastitis, acute, subacute or chronic, is present, but occasionally in an apparently normal organ, and are discharged in the milk. For the most part, they are specifically bovine and are responsible for some 50 per cent. of the bovine mastitis which causes such serious loss to owners of dairy herds. Bovine mastitis may, however, be produced by infection with streptococci of human origin. Clinically this type is indistinguishable from the enzootic form. It is usually subacute, may produce relatively little alteration in the physical, chemical and microscopical character of the milk and may readily escape notice. Hence, when suspicion arises that a human outbreak of streptococcal infection may have been caused by milk, bacteriological investigation is the only means of proof. The first step is the detection of haemolytic streptococci in the milk and for this purpose milk samples from each cow in the suspected herd must be obtained and plated on blood agar. The precautions to be taken and the general considerations to be borne in mind in taking samples are fully discussed by F. C. Minett, A. W. Stableforth and S. J. Edwards (6). In general, they recommend that a sample should consist of about 4 ml. of the fore-milk from each quarter; about 10 ml. of this mixed milk should be centrifuged and plates made from the deposit, the supernatant milk being poured off, except for a few drops in which the deposit can be emulsified. As explained above, surface inoculation of the blood-agar plates in the search for haemolytic streptococci of human type is preferable to the inoculation and pouring of melted medium, although the latter method is rightly preferred by these authors in the study of mastitis as a bovine disease. A satisfactory technique for surface inoculation is as follows: the culture medium (blood agar) is poured some hours previous to inoculation and is carefully dried by incubation of the open inverted Petri dish for a couple of hours in the 37° C. incubator. A drop of the emulsion is then spread on the surface with a sterile right-angled glass rod in such a way as to ensure single colonies at some part of the plate. Suspected colonies may be picked off into blood broth (10 per cent. defibrinated rabbits' blood in nutrient broth) next day. After a night's incubation such blood-broth cultures may be preserved in the refrigerator.

Application of the knowledge acquired by R. C. Lancefield (1) to the serological differentiation of such strains is as yet scarcely possible in ordinary routine epidemiological observations, but the following sketch indicates the lines to follow. The first essential is the preparation by inoculation of appropriate standard cultures of rabbit sera; these should consist of: (1) a serum or sera against strains of human origin (group A), preferably scarlatinal; (2) a serum or sera against strains from bovine mastitis which hydrolyse sodium hippurate and ferment trehalose, but not sorbitol (group B); and (3) a serum or sera against strains from bovine mastitis which are hippurate-negative, and ferment sorbitol but not trehalose (group C). (The small groups D and E have not been serologically studied.) A precipitable extract from the strain to be investigated is prepared by suspending the deposit from 100 ml. of an 18-hour broth culture in saline (2 ml.) with hydrochloric acid to make $N/20$ (blue to congo red paper), immersing the tube in boiling water for 10 min., cooling in

running water and centrifuging. The supernatant fluid is then neutralised with soda, again centrifuged and the water-clear supernatant used for precipitin tests with the sera. The test consists in mixing graded quantities, 0·4, 0·1 and 0·025 ml. of extract (in 0·4 ml. volume) with 0·2 ml. of neat serum, incubating for 2 hours at 37° C. and making final readings for precipitation after storing overnight in the cold. The reactions are sharply group specific; the object of the titration is to ensure that an approximate optimal proportion will be found in one tube. The group specificity depends on the fact established by R. C. Lancefield that the carbohydrate component of streptococci, extracted as above described, is common to all strains, whatever their "type," within each group, and is not represented in strains of the other groups. The groups have the distinctive cultural features already indicated; thus groups A and C are practically indistinguishable on blood agar and are both hippurate-negative, but have opposite fermentation reactions towards trehalose and sorbitol. Group B strains produce typical beta haemolysis in deep colonies but in surface colonies the zone of clarification is very narrow and green pigment is perceptible as a product of alteration of the haemoglobin. Strains of group B grow freely on blood agar containing 40 per cent. ox-bile, whereas those of A and C do not, though C strains will usually grow with 10 per cent. bile and A strains exceptionally. Considerable progress has been made by F. Griffith (5) in the serological analysis of group A for "types," and by his methods precise identification of a group A strain found in suspected milk, with strains from human cases (scarlatina, tonsillitis, etc.), may be attempted with good prospect of success. Similar "types" have been shown by R. C. Lancefield (7) to exist in group B, but these are of interest only in the study of epizootics. No instance is yet on record of human infection with strains of R. C. Lancefield's group B, though a particular type of her group B (O 90) has been identified by Griffith (8) (as "Aronson-Neufeld") in cultures from apparently normal human throats on several occasions. Similarly strains from her group E, to judge from cultural characters only, are not uncommon in the human throat without producing disease and may well have been introduced by milk. Recent work by Colebrook and Hare (not yet published) suggests that group C may occasionally cause human disease.

The specific fibrinolytic power of human haemolytic streptococci offers prospects of a simple laboratory test for their differentiation from bovine strains. Discovered by Tillett and Garner (9), the existence of fibrinolytic activity for human plasma in cultures of *Streptococcus pyogenes* of human origin has been confirmed by Hadfield, Magee and Perry (10), and by Colebrook and Hare (11), though strains of low virulence were found by them to be relatively weak. Human blood (vein puncture) collected over potassium oxalate (0·02 g. to 10 ml. of blood) is centrifuged or allowed to settle overnight and the plasma is then mixed with calcium chloride solution and with the culture of *Streptococcus pyogenes* to be tested (18 hours in meat infusion broth). Plasma 0·2 ml., normal saline 0·8 ml., 0·25 ml. of $CaCl_3$ solution (0·25 per cent. in normal saline) and 0·5 ml. of culture (brought to *p*H 7·2) are incubated together in a water bath at 37° C. for 24 hours. Liquefaction of the clot occurs in from 15 min. to 4 hours with most fibrinolytic strains, provided the human plasma is from a susceptible person, *i.e.* one not recently the subject of a streptococcal infection. The plasma of other animals (except monkeys) is resistant. Tillett and Garner report that all the human strains they tested were fibrinolytic, while all the bovine were not. There was one exception, a bovine mastitis strain (V 10) isolated in association with a human outbreak of streptococcal sore throat and already placed by R. C. Lancefield in group A on serological grounds. "Viridans" streptococci of whatever origin did not digest fibrin. Further epidemiological observations with this test will be of great interest.

No convincing evidence yet exists that any of the other streptococci found in milk are of pathogenic importance for man: those of the *faecalis* group (enterococci) are probably, like the group of *colon* bacilli, normal inhabitants of the intestine both bovine and human. Among other characters for their differentiation is their relatively high resistance to heat, exposure to 60° C. for 10–30 min. acting as a selective procedure for them in mixtures of other streptococci and *colon* bacilli.

II. The *Brucella* infections.

Bacteriological investigation of these may be divided into two sections: (1) the demonstration by (*a*) culture or (*b*) serology of the presence of a *Brucella* infection in animals or man; and (2) the differentiation of the caprine (*melitensis*) from the bovine or porcine strains (*abortus*). As the result of a very large amount of laboratory work in recent years, routine methods can now be laid down with some confidence for both sections.

(1) *Brucella* infections require demonstration, as a rule, only in cows and in human cases of continued fever.

(*a*) The more satisfactory method is, of course, the actual isolation and identification of a culture, since serology may fail to indicate more than that a *Brucella* infection has occurred some time previously. Material from the cow may consist of abortion material (placenta, or foetus or uterine discharges) or of milk; from the human case it would normally be blood or urine, though successful isolations from faeces have been recorded.

General agreement has been reached that Huddleson's (12) medium is the best for primary culture from any of these materials. It consists of liver-infusion—peptone-agar clarified without contact with paper or cotton (by glass-wool filtration or in a mechanical separator), adjusted to *p*H 6·6 and containing 1 in 10,000 gentian violet. There is now also unanimity that for primary culture of *Br. abortus* incubation must be carried out in an atmosphere containing approximately 10 per cent. of CO_2. Smith (13) has described a neat device for the purpose, but most workers have devised suitable arrangements for themselves. Under such conditions it is easy to isolate pure cultures of *Br. abortus* from bovine material by direct plating, the characteristic colonies appearing after incubation for 3 days or less. Even with milk as the material to be examined, isolation by direct plating appears to be easy when the specimens are obtained under clean conditions, though in market milk the presence of other bacteria in very large numbers usually makes direct isolation impossible. Isolation from the blood, *e.g.* in human cases of undulant fever, will generally require preliminary cultivation of the sample, say 5 ml., in a large volume of liver infusion broth (100 ml.). Cultures should be put up in duplicate, one in an atmosphere containing 10 per cent. CO_2, the other in ordinary air; subcultures from these on Huddleson's plates should be made daily till colonies are obtained, or for 14 days or longer. Direct plating of blood is sometimes successful. Urine should be examined by plating the whole of the deposit centrifuged from at least 100 ml. on Huddleson's medium.

Though direct culture is much the more rapid means of diagnosis, inoculation of guinea-pigs may be more certain when the specific bacteria in the material examined are scanty or are accompanied by numerous contaminating microbes. In the former case, intraperitoneal inoculation is to be preferred, in the latter subcutaneous or intramuscular. For market milk, the latter procedure has been widely used of late years and the results have been recently summarised by Pullinger (14). This worker describes a suitable technique which is based on the finding that *abortus* bacteria tend to accumulate in the cream layer. After 2 ml. of gravity cream have

been removed, the centrifuged deposit of 180 ml. of milk (2000 rev. per minute for 30 min.) is mixed with this cream and the mixture inoculated intramuscularly into the thighs of two guinea-pigs. The guinea-pigs are killed after 6 weeks and examined culturally and serologically for evidence of *Brucella* infection. Cultures are made from the organs (spleen, liver, lymphatic glands) as described above; serological tests are made with the heart blood as described below. The use of two guinea-pigs is necessitated by the possibility of premature death of an inoculated animal and by the occasional failure of inoculation with infected material to produce infection.

(*b*) Serological tests depend on the appearance of agglutinins for *Brucella* in the blood serum or in the milk of an infected animal. In performing the test, the primary consideration is the use of "smooth" cultures, *i.e.* cultures which when emulsified in normal salt solution will remain in perfect suspension after heating in boiling water for 2 hours. Satisfactory emulsions both of *Br. abortus* and *Br. melitensis* can be obtained from the Oxford Standards Laboratory. Mixed with equal volumes of graded dilutions of serum (1 in 5, 1 in 10, etc., up to 1 in 2500) they should be incubated in the water bath at 50° C. for 4 hours. A characteristic feature, especially of high-titre sera, is the appearance of a negative prozone in the lower dilutions. This may extend to 1 in 80, and dilutions beyond this should invariably be put up. Much work has been done, especially in America, with rapid agglutination tests in which a drop of a thick suspension of *abortus* bacilli is mixed on a slide with the diluted bovine serum as described by Huddleson and Abell (15). Difficulties, however, are apt to arise in serum of low agglutinin content, as determined by the test-tube method, and the rapid test is not likely to replace the latter in eliminating *Brucella* reactors from infected herds. General agreement can hardly be said to have been reached as to the minimum titre level indicating infection among bovines but, under the conditions outlined above, a titre of 1 in 10 for whey and of 1 in 50 for blood serum should be regarded as evidence of antecedent *Brucella* infection sufficient to justify labelling the animal as a reactor. Many animals giving these titres will fail to yield *Brucella* cultures, however carefully examined, while, on the other hand, an occasional animal excreting *Br. abortus* may present negative or very feeble serum reactions. In attempting to create an *abortus*-free herd, these difficulties have to be met by serological testing at frequent intervals, and by guinea-pig inoculations. Testing for *Brucella* agglutinin in inoculated guinea-pigs is a necessary part of the inoculation test, since its development may be the only positive result, culture failing; a titre of 1 in 10 is significant. It has been observed that mixed market milk containing *Br. abortus* along with an abundance of other organisms may produce no evidence of *Brucella* infection in the inoculated guinea-pig (Pullinger (14)).

(2) The practical value of differentiating between bovine, caprine and porcine strains of *Brucella* lies mainly in the possibility which it offers of tracing the source of human infections. The subject has been reinvestigated with very great care by G. S. Wilson (16) on a basis of 321 strains, and his conclusions are as follows: (*a*) The three main groups have a definite existence correctly indicated by their names, but subgroups occur which vary in one or more respects from the main group into which the majority of their characters would place them; such subgroups tend to be localised geographically and their aberrations from the main groups may reflect response to peculiar environmental conditions. (*b*) In consequence, no single test can be relied upon to allocate individual *Brucella* strains to their proper group. Every strain should therefore be examined for CO_2 sensitiveness, for H_2S formation, for growth in the presence of thionin, basic fuchsin, methyl violet and pyronin and for antigenic structure. (*c*) Any of the three groups may be isolated from man but no typical caprine (*melitensis*) or porcine strain was found in bovine material in Wilson's series. In the application of the tests for dye sensitiveness, Wilson recommends liver

agar of *p*H 6·6 containing thionin 1/60,000 and 1/30,000, basic fuchsin 1/50,000 and 1/25,000, methyl violet 1/100,000 and 1/50,000 and pyronin 1/200,000 and 1/100,000. The dyes used were those of the National Aniline Chemical Company of New York. They were added in half or quarter per cent. solution in distilled water to the liver agar before pouring plates, the dye solution and the melted agar being both at 90° C. at the time of mixing. The plates were inoculated with thick suspensions made by washing off a 2-day liver agar slope in about 0·5 ml. of broth; a control plate without dye was similarly inoculated and all then incubated, either aerobically or, if the strains were CO_2-sensitive, in 10 per cent. CO_2, for 3 days at 37° C. Bovine *abortus* strains were nearly all suppressed by thionin but grew well in the presence of the other three dyes, whereas *melitensis* strains were either not more inhibited, or only slightly more inhibited, by thionin than by the other three; they grew to some extent on all the dye-containing media. Porcine strains, on the other hand, were only slightly inhibited by thionin but their growth was completely suppressed by the other three. Subgroups could be distinguished by increased susceptibility to one or more of the dyes. For *H_2S production* the test familiar to workers on the *Salmonella* group may be used, namely, blackening of lead acetate paper (filter paper soaked in 10 per cent. lead acetate and dried) kept suspended over the medium (liver agar) by fixing a strip with the wool plug. In Wilson's experience the culture had to be incubated for 24 hours at 37° C. (in 10 per cent. CO_2 if necessary) before inserting the test paper, since all *Brucella* strains were liable to produce some H_2S on the first day but no *melitensis* strain produced H_2S on the second day or later. The porcine strains were differentiated into American (H_2S+) and Danish (H_2S-), while the great majority (86 per cent.) of the bovine strains were H_2S+, the exceptions being mostly strains long in cultivation. For *CO_2 sensitiveness* the device of Smith already referred to may be used. Bovine strains, when freshly isolated, either grew better in 10 per cent. CO_2 than in air or failed entirely to grow in air; older strains might not require CO_2, or even grew better in air. *Melitensis* strains all grew in air, though many strains might grow rather better in 10 per cent. CO_2, while porcine strains all grew in air and some were partially or completely inhibited by CO_2. Growth in CO_2 but not in air indicated with practical certainty that the strain was of the bovine *abortus* type. In the *serological testing* of brucellas, a definite advance was made by Wilson and Miles (17) by their use of monospecific serum prepared by absorption with a heterologous type. Some care was necessary in such preparation to ensure that sufficient type-specific agglutinin remained after absorption; not more than 3000 million bacteria per 1 ml. of the diluted serum should be employed, the dilution varying with the original titre from 1/4 with a serum of titre 1/320, to 1/40 with a serum of titre 1/1800. With such monospecific *abortus* and *melitensis* sera Wilson found only one strain, out of 165 examined, which gave an anomalous result; this strain, long in cultivation and of unknown origin but culturally "*abortus*," agglutinated with the monospecific *melitensis* serum. It failed, however, to absorb a *melitensis* serum completely and was probably serologically aberrant. It should be repeated that for all serological work on brucellas it is of prime importance to use "smooth" strains, *i.e.* strains which will show no thermo-agglutination when emulsified in normal saline and heated for 2 hours in a boiling-water bath. "Roughness," according to Wilson, is much more prone to develop during culture of *melitensis* than of *abortus*. Even when already fairly advanced, however, the rough change does not prevent type identification, as sufficient of the type-specific antigen may remain.

Work on brucellas should not be delegated to any but highly experienced laboratory assistants owing to the abnormal risk of infection occurring as the result of careless handling.

III. The *Salmonella* and dysentery infections.

Salmonella infection in man, resulting either in continued fever (enteric or paratyphoid) or in acute gastro-enteritis, after the ingestion of infected milk or milk products, is not uncommon, and extensive outbreaks of both types have been recorded in recent years at home and abroad. The acute gastro-enteritis type may be due to actual infection and disease in the milch cow (enteritis and mastitis)—the outbreak described by Kinloch, Smith and Taylor(18) is a good example—or it may be the result of infection of milk or milk products by a human being suffering from or convalescent from gastro-enteritis due to a *Salmonella* infection acquired in some other way. In this country *Salmonella* infection of bovines with consequent transference to man appears to be less common than it is on the Continent, and is more commonly conveyed by meat than milk. The *Salmonella* types found in bovine infections include both the *Gaertner* group and the *aertrycke* (*typhi-murium*) as well as the type specifically named "*morbificans bovis*." Among the Gaertners, the type "Dublin" may have a specific association with bovines; it has been recognised as the cause of epizootic "calf dysentery" in Denmark, and in many of the human infections from which it has been isolated there is strong suspicion that milk was the source. This Dublin type is peculiar also in its tendency to produce in man continued fever like paratyphoid fever rather than acute gastro-enteritis; hence the source of infection is often difficult to trace. The special human *Salmonella* types (*Salmonella typhi*, *S. paratyphi* A and B) are not known to infect bovines or be conveyed by them, and milk or milk products can acquire them only directly or indirectly (*e.g.* by water) from a human source. The same applies to the dysentery group: there is no evidence that bovines suffer from, or discharge in milk or faeces, any of the several bacterial species producing human dysentery, even though they drink water highly polluted with sewage in which such bacteria are numerous. But milk is readily infected with organisms of the dysentery group during handling and storage and there is good reason to believe that much of the "summer diarrhoea" of infants is, or was, a dysenteric infection conveyed by milk, though actual discovery of the specific bacteria in such milk has rarely been reported.

The general principles of laboratory investigation of the bacteria producing these intestinal infections are well established and, in addition, in both groups the identification of serological types occurring in different outbreaks is relatively easy. Their application to suspected milk or milk products scarcely requires special mention. The selective property which the addition of brilliant green confers on media in the cultivation of all the salmonellas can be used in the search for these in milk. Peptone water containing 1 in 150,000 brilliant green should be put up in large quantities (100 ml. or more) for the addition of small quantities (0·5 ml.) of the suspected milk, incubated for 16–30 hours and a loopful from the supernatant film then plated on MacConkey agar.

Characteristic-looking colonies (pale, structureless) may be then tested by direct emulsification of a speck of the bacterial mass in drops of diluted *Salmonella*-agglutinating sera on a slide and those showing the typical specific clumping may be picked off for complete study by serological and cultural tests. For the typhoid bacillus, the use of brilliant green in selective culture is often less successful, and the bismuth-iron-sulphite agar of Wilson and Blair(19) (by direct surface inoculation) should be employed in addition, though, in practice, the prospects of direct detection of typhoid bacilli in milk, as in water, are limited by the lapse of time which has usually occurred before suspicion falls upon it. No selective medium is known for the dysentery group and there is little prospect of direct demonstration of these bacteria in milk, though the attempt may be made by inoculating numerous plates

of MacConkey or litmus-lactose agar with the centrifuged deposit from it and examining suspicious non-lactose-fermenting colonies by emulsification in diluted anti-Flexner, anti-Sonne, and anti-Newcastle agglutinating sera.

IV. Tuberculosis.

Technical procedures for the detection of tubercle bacilli in milk and milk products comprise (1) direct microscopical examination, (2) culture, and (3) animal inoculation. In all of these general agreement as to methods exists.

(1) Direct microscopical examination is of practical value only for milk in which tubercle bacilli are present in large numbers. It is definitely indicated in the examination of the milk of individual cows when expert veterinary inspection of the udder suggests tuberculous mastitis, since the diagnosis can then be made certain without the delay involved by the other procedures.

The technique of Douglas and Meanwell (20) is generally accepted as superior to other methods for this purpose. Milk (10 ml.) from the suspected udder (or suspected quarter only) is digested for 3 hours at 56° C. with 0·5 ml. of *liquor trypsinae co.* (Allen and Hanbury) in a screw-capped centrifuge tube; after cooling, 5 ml. of ether are added and the tube is violently shaken for at least 3 min.; on centrifugalisation (20 min. at 4000 rev. per minute) the tubercle bacilli collect in a gelatinous film separating the supernatant ether solution of the fat from the clear liquid below and can be detected by the usual staining and microscopical examination of smears made with a loopful of the gelatinous material in distilled water on a slide. In the circumstances mentioned (samples obtained under direct veterinary supervision), the finding of acid-fast bacteria possessing the morphology of the tubercle bacillus is sufficient evidence of tuberculous mastitis for the elimination of such cows; negatives, of course, have no significance unless confirmed by inoculation tests. Samples of market milk will seldom give a direct microscopical finding of tubercle bacilli, in spite of their frequent presence as shown by inoculation, and even the rare positives are subject to reservation since there is always the possibility of their being saprophytic acid-fast bacteria from dust, etc. The same reservation applies in still greater degree to butter. More use, however, might be made of direct microscopical examination of the slime deposited from ordinary milk in separators: large quantities of this material might be digested in antiformin, re-centrifuged and the deposit examined microscopically in the usual way for acid-fast bacteria; the doubt as to their being necessarily specific tubercle bacilli would, however, still remain.

(2) Culture directly from market milk will rarely be attempted, since it is almost impossible to eliminate the common spore-bearing contaminants (*B. mesentericus*, *B. subtilis*, etc.). Maddock (21), however, found that deposits of ordinary milk, treated for 3½ hours at 37° C. with an equal volume of a sodium hypochlorite solution in 15 per cent. NaOH containing a final concentration of 327 parts per million of available chlorine, could be largely freed from resistant spores. Such treatment is known to destroy many tubercle bacilli but some may survive and develop in culture as Maddock, in fact, observed.

But in circumstances such as those described as likely to give good prospects of direct microscopical detection of tubercle bacilli, *i.e.* with skilled supervision of sampling from individual cows, culture might well be employed much more frequently than it is, since the expense is negligible compared with that of inoculation and positive results can be obtained with it at an earlier date. The test shares, though perhaps in a less degree, the defect of direct microscopical examination, namely, that it misses a certain proportion of positives as established by inoculation, *i.e.* it cannot prove a negative, so that the field for its use is restricted. In making culture tests on milk, the samples, drawn preferably by teat-cannula, are centrifuged

and the deposit (from 50 ml.) emulsified in a little 6 per cent. sulphuric or hydrochloric acid; loopfuls of the emulsion are immediately, and at successive intervals of five minutes for 30 min., taken and spread on ordinary coagulated egg slopes (sealed with paraffin wax to prevent drying) which are then incubated at 37° C. for 10 days. The object of taking successive loops, as described, is to obtain specimens of the deposit in which bacteria other than tubercle bacilli have been killed by the acid but in which the tubercle bacilli themselves have not been too devitalised for subsequent growth. Should the culture slopes, examined at the end of 10 days, show no colonies, they should be rubbed over with a platinum loop or spatula before incubation for another week. At the same time a smear from the surface should be examined for acid-fast bacteria. After the further week of incubation, if smears made again with scrapings from the surface show no acid-fast bacteria, the test may be reported as negative.

(3) The inoculation test remains indispensable in detecting tubercle infection in milk and milk products. Certain points of technique are recognised as important and conformity with them would generally be demanded for official tests. These points are (1) that the inoculum available should consist of the deposit from 100 ml. of milk centrifuged for half an hour at 3000 rev. per minute; (2) that half of this deposit emulsified in a few ml. of the supernatant but not treated with antiformin or other disinfectant should be injected, partly subcutaneously, partly intramuscularly, into the thigh of a healthy guinea-pig. The other half of the deposit should be kept in reserve for 3 days in the refrigerator to provide for the case of the animal dying of an acute infection. In this event the remaining half of the deposit may be inoculated intraperitoneally into another guinea-pig, such inoculation being less likely to produce fatal acute infection. Alternatively, two guinea-pigs may be inoculated, subcutaneously and intramuscularly, each with half of the 100 ml. deposit: if both die from an acute infection or otherwise prematurely, another sample of the milk must be obtained. Premature death of the inoculated guinea-pig is a serious problem in the examination of milk from certain districts. As an exceptional measure, treatment of the emulsified deposit with an equal volume of 5 per cent. caustic potash solution for from 15 to 30 min. at 37° C. may be permitted. This treatment destroys most, if not all, of the germs which cause acute infections; the treated emulsion must be neutralised with acid before inoculation. (3) The inoculated animals must be kept under observation for at least 6 weeks, at the end of which period they must be killed and examined; evident positives (enlarged glands) may be killed sooner; proper precautions must be observed to prevent cage infections. (4) On examination a note of the distribution and character of tuberculous lesions should be put on record together with a statement that typical acid-fast bacteria were found in them microscopically.

In the case of butter 100 g. samples are melted in the water-bath at 40° C. and the bottom watery layer containing curd floccules is pipetted off, centrifuged with the addition of a suitable amount of normal saline and the deposit inoculated as in the case of milk. Special care is necessary in performing the autopsies to distinguish lesions of progressive tuberculosis from the local disease due to acid-fast bacilli which may result from the presence of saprophytic acid-fast microbes such as the "butter-bacillus," etc. These, however, can be readily distinguished by culture from the apparent tuberculous lesions since they give evident growth in from one to four days on egg, plain agar or glycerine-agar.

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REFERENCES.

(1) LANCEFIELD, R. C. (1933). *J. exp. Med.* **57**, 571.
(2) MINETT, F. C. and STABLEFORTH, A. W. (1931). *J. comp. Path.* **44**, 114.
(3) GRIFFITH, F. (1927). *J. Hyg.*, Camb., **26**, 363.
(4) BROWN, J. H. (1919). *Monog. Rockef. Inst. Med. Res.* 9.
(5) GRIFFITH, F. (1935). *J. Hyg.*, Camb., **34**, 542.
(6) MINETT, F. C., STABLEFORTH, A. W. and EDWARDS, S. J. (1930). *J. comp. Path.* **43**, 163.
(7) LANCEFIELD, R. C. (1934). *J. exp. Med.* **59**, 441.
(8) GRIFFITH, F. (1935). *J. Hyg.*, Camb. (in press).
(9) TILLETT, W. S. and GARNER, R. L. (1933). *J. exp. Med.* **58**, 485.
(10) HADFIELD, G., MAGEE, V. and PERRY, C. B. (1934). *Lancet*, **1**, 834.
(11) COLEBROOK, L. and HARE, R. (1934). *J. Path. Bact.* **39**, 429.
(12) HUDDLESON, I. F. (1922). *Mich. agric. Exp. Sta. Tech. Bull.* No. 55.
(13) SMITH, J. (1932). *Quart. J. Med.* **1** (N.S.), 303.
(14) PULLINGER, E. J. (1934). *Lancet*, **1**, 967.
(15) HUDDLESON, I. F. and ABELL, E. (1928). *J. infect. Dis.* **42**, 242.
(16) WILSON, G. S. (1933). *J. Hyg.*, Camb., **33**, 516.
(17) WILSON, G. S. and MILES, A. A. (1932). *Brit. J. exp. Path.* **13**, 1.
(18) KINLOCH, J. P., SMITH, J. and TAYLOR, J. S. (1926). *J. Hyg.*, Camb., **25**, 434.
(19) WILSON, W. J. and BLAIR, M. M. (1931). *Ibid.* **31**, 138.
(20) DOUGLAS, S. R. and MEANWELL, L. J. (1925). *Brit. J. exp. Path.* **6**, 203.
(21) MADDOCK, E. C. G. (1933). *J. Hyg.*, Camb., **33**, 103.

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